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THE CHEMISTRY OF THE LIPIDS OF TUBERCLE BACILLI[1]

LXV. An Investigation of Tuberculous Lung Tissue [2,3]

R. J. ANDERSON, R. E. REEVES,[4] M. M. CREIGHTON[5] AND W. C. LOTHROP[5]

It has been shown in a series of investigations conducted in this laboratory during the past several years that the principle ether-soluble compounds occurring in human tubercle bacillus (1), grown on the Long synthetic medium (2), consist of phosphatide (3), acetone-soluble fat (4) and so-called wax (5). All of these substances contain a number of new chemical compounds that are specific and characteristic constituents of the human tubercle bacillus, and which up to the present time have not been found in any other living cells.

The phosphatide contains a new type of glucoside with which the fatty acids are combined. The fatty acids consist of a few ordinary fatty acids, such as palmitic acid, oleic acid and probably a trace of stearic acid, but in addition two new and specific acids are present which have been called tuberculostearic acid (6) and phthioic acid (7). The carbohydrate which contains phosphorus in organic combination yields on hydrolysis with dilute sulfuric acid mannose (8), inositol (9) and a hexose which gives glucosazone on treatment with phenylhydrazine, together with an organic phosphoric acid which appears to be identical with glycerophosphoric acid (3).

The acetone-soluble fat is not a glyceride but consists of fatty acid esters of the disaccharide trehalose (10). The fat contains, in addition to several ordinary fatty acids, relatively large quantities of the specific acids, tuberculostearic acid and phthioic acid (6). Another characteristic constituent is the pigment phthiocol (11).

The so-called wax is a mixture of several compounds, but the principal constituent is an ester of a specific polysaccharide (12) with mycolic acid (13). However, other ether-soluble compounds are liberated on saponification, and especially a levorotatory fatty acid of high molecular weight (14) and the dihydric alcohol phthiocerol (15). Phthiocerol has been found only in the wax of the human and the bovine types of tubercle bacilli (16).

The several specific compounds enumerated above are readily identified by chemical methods, and this is especially the case in regard to tuberculostearic acid, phthioic acid and the pigment phthiocol, all of which occur in the acetone-soluble fat.

Sabin and collaborators (17) have published a series of papers concerning the

[1] From the Department of Chemistry, Yale University, New Haven, Connecticut.

[2] The present report is a part of a coöperative investigation on tuberculosis; it has been supported partly by funds provided by the Research Committee of the National Tuberculosis Association.

[3] This investigation was completed early in 1938 but for various reasons publication of the results has been delayed until the present time.

[4] Holder of a National Tuberculosis Association Fellowship of Yale University, 1936–1937.

[5] Holder of a National Tuberculosis Association Fellowship of Yale University, 1937–1938.

biological reactions of the lipid fractions which we have isolated from the tubercle bacillus. It has been found in these investigations that the compounds when injected into normal animals give rise to cellular reactions similar to the pathological lesions observed in tuberculosis. The phosphatide and phthioic acid were particularly active in stimulating monocytes and epithelioid cells resulting in the formation of typical tuberculous tissue. The cellular reactions were produced in the absence of either dead or living tubercle bacilli.

In view of the results reported from Doctor Sabin's laboratory, it would seem reasonable to assume that the formation of tubercles, as well as caseation, which are typical of the pathological picture in tuberculosis, were due to the action of the lipid compounds mentioned above. In the course of the disease tuberculosis, it would seem probable that large numbers of bacilli eventually disintegrate and the lipid, as well as other cell constituents, such as protein and polysaccharide, are set free and are then able to exert their deleterious physiological reactions. If these assumptions are true, then it might be expected that the specific chemical compounds, which up to the present time have only been found in the tubercle bacillus, would be present in tuberculous lung tissue in sufficient quantities to be recognized by chemical methods.

The isolation from tuberculous human lung tissue of a substance which gives a skin reaction similar to that of tuberculin is mentioned in the book *The Chemistry of Tuberculosis* by Wells and Long (18). It is also mentioned in the same book that substances giving similar reactions have been prepared from serum and urine of tuberculous patients. If the reported observations are true, this would indicate that the highly active protein of the tubercle bacillus, which is responsible for the skin reaction, is present in recognizable amount in the tissues and fluids of patients suffering from tuberculosis. However, the evidence is not entirely clear.

A number of investigations dealing with the chemical composition of the lipids occurring in tuberculous lung tissue and in caseous material have been published, and an excellent review of this earlier work is given by Long (18).

The present investigation was undertaken with the special object of attempting to isolate from human tuberculous lung tissue certain of the specific lipid constituents which we have found previously to occur only in human tubercle bacilli. For this work we were provided, through the coöperation of Dr. E. R. Long of The Henry Phipps Institute, Philadelphia, Pennsylvania, with a large quantity of tuberculous and caseous material from human lung tissue, obtained on autopsies of patients who had died of tuberculosis. The material had been carefully selected and nontuberculous tissue had been removed. The lipids were extracted and examined by the methods that we have developed in the investigation of the lipids of the tubercle bacillus.

The first substance that we tried to identify was the pigment phthiocol. We have previously shown that very small amounts, even fractions of a milligram of phthiocol, can be determined by a colorimetric method (19). The crude lipids obtained from the tuberculous lung tissue did not yield a trace of phthiocol.

The next group of substances sought for was the specific liquid saturated fatty acids, tuberculostearic acid and phthioic acid, which constitute nearly 40 per

cent of the total fatty acids of the acetone-soluble fat of the human tubercle bacillus. The results were again completely negative. We were unable to isolate a trace of these acids from the acetone-soluble fat prepared from tuberculous lung tissue.

In view of the negative results obtained in this investigation the question was considered whether it would be possible to isolate small amounts of phthiocol, tuberculostearic acid and phthioic acid that had been incorporated with a large quantity of fresh normal lung tissue. Small amounts of the compounds mentioned above were sent to Dr. E. R. Long, and they were incorporated with minced normal human lung tissue. The material was then lyophilized and returned to this laboratory for analyses. We found that the added substances could be isolated and identified without any difficulty. It is evident, therefore, that phthiocol, tuberculostearic acid and phthioic acid are stable chemical compounds that are not destroyed or changed in contact with normal lung tissue.

The specific polysaccharide of the human tubercle bacillus can be detected in very high dilutions by means of the precipitin reactions with immune serum. Even crude polysaccharide preparations give precipitin reactions in dilutions greater than one part per million. In order to determine whether tuberculous lung tissue contained any of this specific substance, a crude polysaccharide was prepared from this material and tested against immune serum, but the product gave no precipitin reaction whatever.

It may be concluded from the negative results obtained in this investigation that the actual amounts of tubercle bacilli and their break-down products that may be present in tuberculous lung tissue are insignificant in comparison with the weight of the diseased tissue. Although it is well known that tuberculous tissue is highly infectious when inoculated into susceptible animals, our results would indicate that the specific chemical bacillary products are not present in sufficient amounts to be detected by chemical methods. On the other hand, our negative results might indicate that tubercle bacilli growing in living tissue do not produce the same characteristic chemical compounds that are found in bacilli that have been cultivated on an artificial medium. Whatever the explanation may be, we can only record that it was impossible to detect in tuberculous lung tissue a trace of any of the chemical compounds that are characteristic constituents of the tubercle bacillus when cultivated on an artificial medium.

EXPERIMENTAL

The material used in the present investigation was received from The Henry Phipps Institute, Philadelphia, Pennsylvania, during November and December, 1936. Eight one-gallon bottles were received containing a total of 2,225 g. of moist tuberculous lung tissue. Each bottle contained from 200 to 475 g. of tissue mixed with from 2 to 3 liters of 95 per cent alcohol. According to the autopsy findings the material had been obtained from cases of far advanced pulmonary tuberculosis, but included 3 cases described as miliary tuberculosis. The contents of the bottles varied distinctly in appearance. One bottle contained reddish colored material. In two of the bottles the contents were light gray or nearly white, while the others contained small dark colored lumps, some of which were black in color.

The bottles were shaken occasionally and allowed to stand at room temperature until

January 12, 1937, when the supernatant solutions were siphoned off, combined and saved. The tissue residue was next transferred to a five-gallon bottle and further dehydrated with 10 liters of alcohol. After standing for about three days with occasional shaking, the clear supernatant solution was siphoned off and combined with the first alcoholic extract. This solution was worked up as will be described later.

The tissue was next forced through a fine meat grinder, after which it was extracted with three liters of alcohol-ether, equal parts, with occasional shaking at room temperature for three or four days. The solvent was then siphoned off and the extraction was repeated with the same mixture of solvents and warming at 55°C. under a reflux for three hours. After the mixture had cooled to room temperature, the solvent was siphoned off and combined with the first alcohol-ether extract.

After the extraction mentioned above, the tissue residue was extracted with 2 liters of chloroform by warming to 55°C. for three hours. After the mixture had cooled to room temperature, the solvent was siphoned off and the extraction was repeated with three liters of chloroform-ether, equal parts. The tissue residue was then filtered off on a Büchner funnel and washed with chloroform. The tissue residue after it had been dried in air formed a hard cake which weighed 362 g. The chloroform extracts were evaporated to dryness under reduced pressure and left a residue of lipid material which weighed 2.99 g.

Recovery of lipids from the alcohol extract: The combined alcoholic extracts, about 6 gallons, were concentrated under reduced pressure to a volume of about 2 liters. The concentrate was diluted with water and thoroughly extracted with ether. The ethereal solution was dried over sodium sulfate, filtered and the ether was distilled off. The residue after it had been dried in a vacuum desiccator formed a brownish fat-like mass and it weighed 37.9 g.

The aqueous solution was examined as will be described later.

Lipid from the alcohol-ether extracts: The combined alcohol-ether extracts were concentrated under reduced pressure and the residue after dilution with water was extracted with ether. The ethereal extract was dried over sodium sulfate, filtered and evaporated to dryness, yielding 12.3 g. of lipids.

The aqueous solution was combined with the aqueous solution from the alcoholic extract.

Examination of the aqueous solution: The combined aqueous solutions, from which the lipids had been removed by extraction with ether as mentioned above, measured 6,275 cc. Of this solution 10 cc. were evaporated to dryness leaving a residue which weighed 42.5 mg., corresponding to 26.6 g. of total solids, consisting of carbohydrates and other water-soluble material.

Test for serological activity in the water-soluble compounds: 10 cc. of the above mentioned aqueous solution were concentrated under reduced pressure nearly to dryness and the residue was dissolved in 5 cc. of saline solution and made up to 10 cc. with water. This solution, as well as dilutions of 1:10 and 1:50 were tested against immune horse serum with completely negative results, thus indicating that the aqueous solution did not contain a detectable amount of the specific polysaccharide which is present in the tubercle bacillus.

Examination of the lipid fractions: The lipid fractions mentioned above were combined, giving a total weight of 53.2 g. The material was treated with 150 cc. of ether, giving a cloudy solution. Addition of 300 cc. of acetone caused a precipitation of a sticky mass that adhered to the bottom of the flask. After the mixture had stood over night the clear supernatant solution was decanted; the precipitate was again dissolved

in 100 cc. of ether, reprecipitated with 200 cc. of acetone and the supernatant solution was decanted. The precipitate was treated with 100 cc. of ether and the insoluble material was removed by centrifugation, washed with ether and dried in a vacuum desiccator. The substance was a wax-like brittle solid and it weighed 4.4 g. This fraction which evidently consisted of sphingomyelin and cerebrosides was not further examined.

The ethereal solution was concentrated to a volume of about 100 cc. and 200 cc. of acetone were added. After the precipitate had settled the supernatant was decanted and combined with the previous ether acetone mother liquors.

The acetone-insoluble substance which represented crude phosphatide weighed 17.9 g. after it had been dried in a vacuum desiccator. This fraction was not further examined.

The ether-acetone solutions from which the phosphatide had been precipitated were combined, concentrated until the ether was removed and then cooled in ice water. A solid precipitate separated and was filtered off, washed with cold acetone and dried in a vacuum desiccator. This substance weighed 6.1 g.

The lipids contained in the cold acetone were recovered by evaporation of the solvent to dryness. The material formed a partly crystalline mass which weighed 24.5 g. This fraction represents the acetone-soluble fat.

The operations just described yielded the following lipid fractions which are summarized below.

		Per cent of total lipids
1. Ether-insoluble fraction	4.4 g.	8.27
2. Phosphatide fraction	17.9 g.	33.64
3. Acetone-insoluble fraction	6.1 g.	11.46
4. Acetone-soluble fat	24.5 g.	46.05
Total recovered lipids	52.9 g.	99.42

According to past experience the characteristic substances of the tubercle bacillus which we were seeking should be concentrated in acetone-soluble fat. For this reason the first three fractions were not analyzed.

Examination of the acetone-soluble fat: The most easily recognizable characteristic constituents, namely phthiocol, tuberculostearic acid and phthioic acid, of the tubercle bacillus occur in the acetone-soluble fat. The acetone-soluble fat was, therefore, saponified and the cleavage products were separated and examined as described below.

For saponification 20 g. of the acetone-soluble fat were saponified by refluxing for five hours with 200 cc. of 5 per cent alcoholic potassium hydroxide. The soap solution was concentrated to a volume of 100 cc., diluted with 600 cc. of water and extracted four times with ether for the removal of unsaponfiable matter. The ethereal extracts were combined, washed with water, dried over sodium sulfate, filtered and evaporated to dryness. The unsaponifiable matter was a crystalline solid consisting of crude cholesterol and it weighed 6.2 g. corresponding to 31 per cent of the fat.

Purification of cholesterol: The crude cholesterol was dissolved in ether and the solution was decolorized with norit, after which the ether was distilled off. The residue was twice recrystallized from alcohol, yielding colorless, beautifully crystalline plates, which weighed 3.6 g. The crystals gave the Liebermann-Burchard reaction and melted at 147 to 148°C. There was no depression of the melting point when the crystals were mixed with pure cholesterol which melted at the same temperature.

The mother liquors on concentration to dryness left a slightly yellowish crystalline

residue which weighed 2.5 g. The results show that cholesterol was the principal constituent of the unsaponifiable matter.

Isolation of the fatty acids and examination for the presence of phthiocol: The alkaline soap solution after the unsaponifiable matter had been removed was acidified with hydrochloric acid and extracted with ether.

The aqueous solution was saved and examined for glycerol as will be described later.

The ethereal extract was washed with water, after which it was extracted with 1 per cent sodium bicarbonate solution for the removal of any phthiocol that might be present. The bicarbonate extract which was of brown color was acidified with hydrochloric acid and extracted with ether, which removed practically all of the coloring matter. The ethereal solution after it had been washed with water was concentrated nearly to dryness and transferred to a distilling flask. The solution was diluted with water, acidified with sulfuric acid and distilled with steam. The distillate was collected in a receiver, containing a dilute solution of sodium bicarbonate. In case phthiocol had been present, the distillate would have shown a red color because phthiocol is volatile in steam and it gives bright red colored alkali salts. Even after 250 cc. of distillate had been collected, the solution was perfectly colorless. It is evident, therefore, that the acetone-soluble fat did not contain a trace of phthiocol.

Isolation of the fatty acids: The ethereal solution after it had been extracted with sodium bicarbonate was washed with water, dried over sodium sulfate, filtered and the ether was distilled off. The residue formed a solid semicrystalline mass of fatty acids which weighed 11.9 g. The acids possessed a very unpleasant odor.

Examination of the aqueous solution from which the fatty acids had been extracted—Isolation of glycerol: The acidified aqueous solution from which the fatty acids had been extracted was tested for reducing sugars, both before and after it had been boiled with dilute hydrochloric acid. The results were entirely negative, thus showing that the solution did not contain either reducing sugars or polysaccharides. After these tests had been made the solution was neutralized with sodium carbonate, evaporated to dryness under reduced pressure and the residue was extracted with pyridine. The pyridine solution was evaporated to dryness under reduced pressure and left a syrupy residue weighing 0.83 g. A portion of the residue was heated with acid potassium sulfate and gave a strong reaction for acrolein. The result would indicate that the syrup was glycerol.

Examination of the fatty acids: The total fatty acids were converted into lead salts and the latter were treated with ether. The ether-insoluble lead salts were filtered off and washed carefully with ether. The ether-insoluble and the ether-soluble lead salts were decomposed with dilute hydrochloric acid in the usual manner and the free acids were recovered. The ether-insoluble lead salts yielded 3.6 g. of white crystalline fatty acids, while the ether-soluble lead salts gave 8 g. of a yellowish mobile oil.

Examination of the solid crystalline fatty acids: The solid fatty acids were dissolved in 15 cc. of warm acetone. The solution solidified to a white crystalline mass on cooling to room temperature. The crystals were filtered off, washed with cold methyl alcohol and dried *in vacuo* and weighed 2.5 g. The acid consisted of white, thin crystalline plates and melted at 55 to 56°C. The molecular weight determined by titration was 265.9. Judging by these properties, the solid acids appeared to be a typical mixture of palmitic and stearic acids.

Examination of the liquid fatty acids: The liquid fatty acids had an iodine number, Hanus, of 106.7. In order to determine whether any liquid saturated fatty acids such as tuberculostearic acid and phthioic acid were present, the balance of the liquid acids, about 7.8 g., was reduced in alcoholic solution with hydrogen in the presence of platinum oxide.

After the catalyst had been filtered off, the solution was neutralized with potassium hydroxide, concentrated to a volume of about 25 cc., diluted with water and poured into an aqueous solution of lead acetate. The resulting lead salt was filtered off, washed with water and dried in a vacuum. The dried lead salt was treated with ether and the insoluble lead salt was filtered off, washed with ether and decomposed by shaking with dilute hydrochloric acid, after which the solid reduced acid was isolated in the usual manner. The ethereal solution and washings were combined and examined, as will be described below.

Examination of the solid reduced acid: The reduced acid formed a nearly white crystalline solid and weighed 7.3 g. Crystallization from 30 cc. of acetone yielded 5.7 g. of white, irregular plates which melted at 64 to 65°C. The molecular weight determined by titration was 288.5. The data would indicate that the acid consisted largely of stearic acid but a small amount of some higher acid was probably present. No attempt was made, however, to separate this mixture into pure constituents.

Examination of the ether-soluble portion of the lead salts: The ethereal solution obtained on treating the lead salts with ether was washed with dilute hydrochloric acid and water, after which it was dried over sodium sulfate and evaporated to dryness. A crystalline residue remained which weighed 0.24 g. This material should have contained tuberculostearic acid and phthioic acid if these acids were present in the liquid fatty acid fraction, or it might consist of unsaponifiable matter which had not been removed completely during the extraction of the saponification mixture. The substance was found to be optically active $[\alpha]_D$ in chloroform $= -11.7°$. The molecular weight determined by titration was 1,637, thus indicating that the substance consisted mainly of neutral material. The titrated solution which contained all of the above substance was made strongly alkaline with potassium hydroxide, diluted with water and extracted with ether. The ethereal extract on evaporation to dryness yielded 0.18 g. of a white crystalline solid, which on recrystallization from 5 cc. of alcohol gave 66.7 mg. of large, thin, colorless plates. The crystals melted at 141 to 143°C. and gave a distinct and typical Liebermann-Burchard reaction. These results indicated that the neutral material was a mixture of cholesterol and dihydrocholesterol. The low levorotation and the low melting point bear out this assumption. It is well known that cholesterol is reduced rather slowly to dihydrocholesterol, hence it is not surprising that after catalytic reduction sufficient unchanged cholesterol remained to give a positive Liebermann-Burchard reaction.

The presence of cholesterol in the liquid acid fraction needs a word of explanation. The original saponification mixture had been extracted only four times with ether for the removal of unsaponifiable matter, but it should be mentioned that four extractions are insufficient for the complete removal of sterols from a saponification mixture.

Examination of the alkaline solution after the above mentioned neutral material had been removed: The alkaline solution was acidified with hydrochloric acid and extracted with petroleum ether. Petroleum ether was used in preference to ordinary ether in this case because the solution contained phenolphthalein, and the latter is readily extracted from acidified aqueous solution with ether, but it is insoluble in petroleum ether. On evaporation of the solvent there remained a white solid crystalline acid which weighed 52 mg. Since the lead salts of ordinary saturated fatty acids possess a slight solubility in ether, it is not surprising that a small amount of the solid reduced acids was present in ether solution.

From the results obtained it is evident that neither tuberculostearic acid nor phthioic acid were present in detectable amounts in the liquid acid fraction.

Further examination of the lung tissue for phthiocol and fatty acids: In order to determine

whether the extracted lung tissue residue contained phthiocol or appreciable quantities of lipids, 10 g. of the material were refluxed for six hours with 100 cc. of 10 per cent aqueous sodium hydroxide. The mixture was filtered to remove some black insoluble material. The clear filtrate which was of brownish red color was acidified with hydrochloric acid and extracted with ether. The ethereal extract was shaken with dilute sodium bicarbonate solution. The bicarbonate extract which was of light brown color was acidified with sulfuric acid, distilled with steam, and the distillate was collected in a receiver which contained a dilute solution of sodium bicarbonate. The distillate remained perfectly colorless. It is evident, therefore, that 10 g. of the extracted lung tissue did not contain a detectable amount of phthiocol.

Examination for fatty acids: The ethereal extract after treatment with dilute sodium bicarbonate was dried over sodium sulfate, filtered and evaporated to dryness. The residue possessed an exceedingly unpleasant odor and it weighed only 20 mg. This result shows that the extracted tissue residue did not contain any appreciable quantity of lipids.

Further investigation of tuberculous lung tissue: In view of the negative results obtained in the investigation described above, a new lot of lyophilized tuberculous human lung tissue was received from The Henry Phipps Institute. The material which weighed 33.2 g. was extracted with alcohol-ether and with chloroform as described before.

The total lipids amounted to 5.0697 g., corresponding to 15.26 per cent of the lyophilized tissue. Cholesterol was determined by the digitonin method on the crude lipid, and it was found to contain 15.75 per cent of total cholesterol and 9.83 per cent of free cholesterol. The lipids were separated as described before into the following four fractions.

A—Ether insoluble material 0.5330 g.
Analyses, P 2.80, N 2.90 per cent
B—Phosphatide 1.5267 g.
Analyses, P 3.24, N 2.87 per cent
C—Acetone insoluble material 0.30 g.
D—Acetone soluble fat 2.4523 g.

Examination of the phosphatide: Of the phosphatide, 1.43 g. were hydrolyzed by refluxing with 5 per cent aqueous sulfuric acid and the cleavage products were separated in the usual manner. The fatty acids weighed 0.80 g. and were combined with the fatty acids obtained from the acetone soluble fat and examined for liquid saturated acids as will be described below.

The water-soluble constituents consisted of glycerophosphoric acid which was isolated as the barium salt. Choline was also present and was isolated as the chloroplatinate. No reducing sugar was present.

Examination of the acetone soluble fat: The acetone-soluble fat and the acetone-insoluble fraction were combined and saponified by refluxing with alcoholic potassium hydroxide. The unsaponifiable matter was removed by six extractions with ether and was found to consist of cholesterol, which after recrystallization from alochol was obtained as colorless plate-shaped crystals which melted at 146 to 147°C. The crystals gave the Liebermann-Burchard reaction.

The fatty acids weighed 1.6 g. and were combined with the fatty acids obtained from the phosphatide. The mixed acids were examined for phthiocol but not a trace was found. The mixed acids were reduced with hydrogen in the presence of platinum oxide, after which they were converted into lead salts and the lead salts were treated with ether. The ethereal solution which should contain ether-soluble lead salts was shaken with dilute hydrochloric acid, washed with water and evaporated to dryness. A solid crystalline

residue was obtained that weighed only 4 mg. It seems safe, therefore, to conclude that no saturated liquid acids characteristic of the tubercle bacillus were present in the lipids that were examined.

Examination of the defatted lung tissue for serological activity: 10 g. of the defatted tuberculous lung tissue were ground carefully in a mortar with 65 cc. of saline solution, after which the insoluble tissue was filtered off and washed with saline. A second extraction with 25 cc. of saline was made in the same manner. The combined extracts were filtered through a layer of washed asbestos. The clear filtrate was adjusted to pH 7 and made up to a volume of 70 cc. An aliquot of the solution on concentration to dryness and after drying *in vacuo* to constant weight was found to contain 40.7 mg. of solids per cc.

The solution when tested against immune horse serum gave a weak but positive precipitin reaction. However, dilutions higher than 1 to 10 gave no precipitin reaction.

In order to confirm these results another 10 g. portion of the defatted lung tissue was extracted with saline as described above. In this case positive weak precipitin reactions with immune horse serum were obtained with the concentrated solutions but not with dilute solutions.

Examination of normal lung tissue for serological activity. One lot of lyophilized normal human lung tissue was received from The Henry Phipps Institute and it was extracted as described above. The defatted tissue was ground in a mortar with saline in the same manner as mentioned above, and the filtered extract was tested against immune horse serum. In this case no precipitin reaction was obtained even with concentrated solutions.

The weak positive precipitin reactions observed with the concentrated saline extracts of tuberculous human lung tissue might indicate the presence of traces of the specific tubercle bacillus polysaccharide. However, the results cannot be considered conclusive.

Examination of normal human lung tissue to which had been added phthiocol, tuberculostearic acid and phthioic acid: In order to determine whether the specific products of the human tubercle bacillus metnioned above could be isolated and identified after being incorporated with normal human lung tissue, the following experiment was carried out. At the suggestion of Dr. E. R. Long we sent to The Henry Phipps Institute 0.1 g. synthetic phthiocol, 2.5 g. crude tuberculostearic acid and 2.5 g. crude phthioic acid. These substances were mixed with minced normal human lung tissue, after which the material was lyophilized and returned to this laboratory for analyses. The extraction of the lipids was carried out as described above by means of alcohol-ether and chloroform. Unfortunately a portion of the lipids was lost during the isolation, which rendered a quantitative recovery of the added substances impossible.

The total crude lipid weighed 10.3 g. and was saponified with alcoholic potassium hydroxide. The cleavage products were separated into unsaponifiable matter and fatty acids as described before. The unsaponifiable matter consisted of cholesterol, which after crystallization from alcohol melted at 148 to 149°C. There was no depression of melting point on admixture with pure cholesterol.

Isolation of phthiocol: After removal of the unsaponifiable matter the soap solution was acidified with hydrochloric acid and extracted with ether. The ethereal extract which was a deep yellow color was extracted with 0.5 per cent aqueous sodium bicarbonate. The alkaline extracts were of deep red color and the extraction was continued until the extracts were colorless. The phthiocol was isolated from this extract in the usual manner and recrystallized from methyl alcohol. The crystalline phthiocol weighed 62 mg. and it had a correct melting point 173 to 174°C. The phthiocol remaining in the mother liquor was estimated by the colorimetric method described by Reeves and Anderson (19), and amounted to 6.6 mg. The recovery of phthiocol was, therefore, 68.6 per cent.

Isolation of tuberculostearic acid and phthioic acid: In order to isolate the tuberculostearic acid and phthioic acid in one operation, the mixture of fatty acids was reduced with hydrogen in the presence of platinum oxide, after which the fatty acids were separated by the lead soap ether procedure. The solid crystalline acids obtained from the ether insoluble lead salts weighed 4.12 g. and were not further examined.

The liquid saturated fatty acids isolated from the ether soluble lead salts formed an oily liquid at room temperature and weighed 3.10 g. The specific optical rotation of the liquid acids in alcoholic solution was +1.76°. No attempt was made to separate these acids into pure components but the dextrorotation was a clear indication that phthioic acid was present.

SUMMARY

1. Tuberculous human lung tissue has been examined for certain specific chemical compounds, namely, phthiocol, tuberculostearic acid, phthioic acid and the specific polysaccharide which occur in the human tubercle bacillus when grown on a synthetic medium.

2. It was impossible to detect even traces of the specific substances mentioned above in the tuberculous lung tissues that were examined.

3. Phthiocol, tuberculostearic acid and phthioic acid incorporated with normal human lung tissue could be isolated and identified, thus showing that these compounds are not destroyed by lung tissue.

SUMARIO

1. En este estudio examinóse tejido pulmonar tuberculoso humano, en busca de ciertos específicos compuestos químicos, a saber, *phthiocol*, ácido tuberculoesteárico, ácido *phthioic*, y el poliosacárido específico que se encuentra en el bacilo tuberculoso humano al cultivarlo en un medio sintético.

2. Resultó imposible descubrir ni siquiera indicios de las precitadas substancias específicas en los tejidos pulmonares estudiados.

3. En cambio, pudieron aislarse e identificarse, *phthiocol*, ácido tuberculoesteárico y ácido *phthioic* incorporados en el tejido pulmonar normal humano, demostrando que dicho tejido no destruye dichos compuestos.

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THE CULTURING OF TUBERCLE BACILLI FROM THE HUMAN SPLEEN, LIVER AND KIDNEY[1, 2]

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While numerous attempts have been made to recover tubercle bacilli from human lymph nodes and pulmonary tissue, there have been relatively few studies of the dissemination of tubercle bacilli in the other organs of the body. Nolan (1), in analyzing 683 cases of tuberculosis, found tubercles in the spleen in 9 per cent and in the liver in 8 per cent of the cases. These figures apparently refer to grossly demonstrable tubercles, for Long and Faust (2), by a careful study of histological sections from 102 cases of pulmonary tuberculosis, were able to demonstrate tubercles in the spleen in 69 per cent of the cases, in the liver in 60 per cent and in the kidney in 23 per cent.

Not satisfied with determining merely the presence or absence of tubercles or tubercle bacilli, Lurie (3), working with infected guinea pigs and rabbits, devised a method for determining the *number* of bacilli in a given organ. After some experience in this laboratory with Lurie's technique, as applied to guinea pigs, it was determined to make a similar study of human viscera.

Tissues for culture were obtained from 186 patients who died at the William H. Maybury Sanatorium. These were unselected autopsies, most of them being consecutive. The age of the patients at time of death varied from two to seventy years, 13 of the group being under fifteen years of age. In the great majority of the 186 cases death was due to pulmonary tuberculosis. However, as will be mentioned later, cardiovascular-renal disease was the important factor in causing death in a number of cases. At first the spleen was the only organ cultured. Then the liver was included in the study, and finally the kidney.

METHOD

In each case the surface of the organ to be cultured was seared and, with sterile instruments, a wedge-shaped piece of tissue was removed by incising through the seared area. Then the tip of this wedge was cut off for culturing, thus excluding the seared portion of the wedge. Next the selected bit of tissue was weighed in a sterile weighing bottle, the attempt being made to include approximately 0.1 g. of tissue for culturing. The weighted tissue was then triturated with sterile mortar and pestle and the material diluted with sterile saline to a volume of 5 cc. Half of this tissue suspension was treated with hydrochloric acid and sodium hydroxide to destroy any pyogenic bacteria which might happen to be present in the organ. Then measured amounts of both treated and untreated suspensions were planted at various dilutions on Löwenstein's media, 6 tubes of media being used for each dilution.

The tubes were examined at intervals of two weeks and the colonies of tubercle bacilli which appeared were counted. From the total colony count, knowing the proper dilution

[1] Presented in part at a session of the Mississippi Valley Sanatorium Association, St. Paul, Minnesota, October 4, 1940.

[2] From the Wm. H. Maybury Sanatorium (Detroit Municipal Tuberculosis Sanatorium), Northville, Michigan.

factor, it was possible to estimate the number of tubercle bacilli per gram of tissue in the organ. The maximum count obtained, whether it was found in the treated or the untreated material, was taken as representative of the organ. Thus if, as frequently happened, only a single colony appeared in the lowest or 1:10 dilution of the 0.1 g. of tissue, this was taken as indicating that the parent organ contained approximately 100 tubercle bacilli per gram of tissue.

SPLEEN CULTURES

Colonies of tubercle bacilli were found in 116, or 62 per cent, of the 186 spleen cultures. This compares with 69 per cent of spleens found positive for *tubercles* by microscopical examination in the series of Long and Faust (2). The latter study, however, was based upon 126 cases of "fatal pulmonary tuberculosis," while our own series included 29 cases in which cardiovascular-renal disease was a primary factor in causing the death of the patient. Four of these cases showed no sign of active tuberculosis at the postmortem table. In every one of these 29 cases, spleen cultures were negative for tubercle bacilli. If these cases are excluded the proportion of positive spleen cultures in the remaining 157 cases becomes 74 per cent.

The number of colonies which appeared in different spleen cultures varied greatly. As just mentioned, a large number of the cultures were completely sterile. Eleven of the cultures showed only a single colony of tubercle bacilli while in 26 cases the indicated content of tubercle bacilli was 500 or less per gram of tissue. At the other extreme, some of the spleens had an indicated bacillary content of many thousands of bacilli, the maximum count for any one spleen indicating 860,000 tubercle bacilli per gram of tissue.

CORRELATION BETWEEN CULTURAL AND HISTOLOGICAL STUDIES

In our own series a single histological section from each organ was studied for pathological changes before results of the spleen cultures were available. When the results from the histological and cultural studies were finally compared, those histological sections which failed to corroborate the cultural findings were reexamined to make sure that no tubercles had been missed. As a result of this double check of the slides, tubercles were found in the sections of all but 15 of the 116 spleens with positive cultures. None of the spleens with negative cultures showed tubercles in the histological section. The 15 cases mentioned above fell into three groups as far as the histological picture was concerned:

1: Sections from 5 of the spleens showed no abnormality. In these 5 cases all of the corresponding bacillary counts were 500 or less per gram of splenic tissue. Presumably in these cases the examination of additional sections would have revealed tubercles.
2: In 8 cases with positive culture the corresponding spleen sections showed extensive amyloid disease. These cases had bacillary counts varying from 400 to 97,000, which indicates that in cases complicated by marked amyloidosis tubercle bacilli may be present (probably in the amyloid itself) without exciting the characteristic tissue response.
3: Finally, in 2 cases, each with bacillary counts above 10,000, a diffuse proliferation of epithelioid cells throughout the splenic pulp was encountered.

The *type* of tubercle appeared even more important than their number in a given section in determining how many tubercle bacilli one might expect to find by culturing the spleen. The presence of just a few proliferative tubercles in the section was associated with a low count, while in cases with very high counts, soft, caseous tubercles were usually found. This correlation was so striking as to give the impression that a single proliferative tubercle would probably give rise to only a single colony, while a caseous, miliary tubercle might yield hundreds or thousands of colonies. One case, which had a bacillary count of 257,000 per gram of tissue, showed 250 minute tubercles in a single section of the spleen. On the other hand, the case with the maximum bacillary count of 860,000 had only 7 tubercles in the corresponding spleen section, all of these being of the soft, caseous variety.

COMPARISON OF LIVER AND SPLEEN CULTURES

In 117 cases cultures were made from both liver and spleen, the liver culture being found positive in 59 instances and the spleen culture in 72 instances (table 1).

TABLE 1

Comparison of cultures of liver and spleen

	NUMBER OF CASES
Both spleen and liver cultures positive	51
Liver culture positive, spleen culture negative	8
Spleen culture positive, liver culture negative	21
Both cultures negative	37
Total	117

In most of the cases the indicated number of tubercle bacilli in the spleen, per gram of tissue, was ten to twenty times as great as the number in the liver. However, in addition to the 8 cases of positive liver and completely negative spleen cultures, there were 9 cases in which, both cultures being positive, the indicated bacillary content of the liver was greater. Thus 17 cases were found in which the liver showed more tubercle bacilli per gram than did the spleen. In every one of these 17 cases extensive tuberculous disease was found in the abdominal cavity. Tuberculous enteritis was the complication most frequently encountered, though peritonitis and tuberculosis of the mesenteric lymph nodes were also found in some cases. The fact that disease in the abdominal cavity was present whenever the liver contained more tubercle bacilli than the spleen indicates that in these cases tubercle bacilli were gaining access to the portal circulation and were being filtered out of the portal blood by the liver. One case was particularly instructive from this point of view.

Case Report

M. S. 282, a white male, forty-two years of age, was admitted to Maybury Sanatorium with the history of weakness, productive cough and progressive loss of weight of three

months' duration. The patient, born in Ireland, had worked in Detroit nineteen years as a chauffeur. There was no known exposure to tuberculosis. Death occurred one month after admission.

Postmortem examination revealed miliary tubercles throughout both lungs, along with small apical cavities in both right and left lungs. There was no ulceration of the intestines, but the midportion of the mesentery contained a round node, 2.5 cm. in diameter, with soft, buttery, caseous material in its centre, and a partly calcified, eggshell capsule. Cultural studies revealed *no* tubercle bacilli in the spleen but there were 22,000 tubercle bacilli per gram of liver tissue.

In this case there can be little doubt that the caseous mesenteric node, the result of childhood tuberculous infection, had broken down and was discharging infectious material into the portal circulation. The fact that the patient was born in Ireland fits in with this hypothesis, because of the known high incidence of intestinal tuberculosis in the British Isles (4). If one accepts this interpretation of the findings it is only reasonable to assume that the entire picture in this case, including the pulmonary disease, was the result of endogenous reinfection from the mesenteric node.

Comparing the pathological histology of the liver with the cultural studies of the same organ yielded results similar to those noted in the spleen. In the 59 cases with positive liver cultures, tubercles were found in the corresponding sections in all but 14 cases. In 4 of these cases, all with low bacillary content, no pathological change was noted. In another 4 cases extensive amyloid disease was found, in 6 cases marked fatty change about the portal triads was noted.

CULTURES OF THE SPLEEN, LIVER AND KIDNEYS

In 87 cases cultures of the three organs, spleen, liver and kidneys, were made. These cultures were positive in the case of the spleen 63 times, the liver 48 times and the kidney 24 times. In no case did the kidney tissue contain more bacilli than tissue of the corresponding spleen, but there were 11 cases in which the bacillary content of the kidney was greater than that of the liver.

The correlation between positive cultures and the finding of tubercles in the corresponding histological sections was less in the case of the kidney than was noted with either of the other organs. Among the 24 cases which showed positive kidney cultures, tubercles were found in only 10 cases. In 4 cases no pathological change was noted. In 2 cases amyloid disease was pronounced, while in 8 cases small renal scars were found. It is our impression that, in the tuberculous patient, these renal scars may be the sole residuum of tubercle bacilli which have reached the organ. One case in this series was of particular interest.

Case Report

M. S. 480, a white man, aged sixty-nine, with well controlled pulmonary tuberculosis of ten years' duration, rather abruptly developed fever and gradually increasing dyspnoea. Death occurred eight weeks after his temperature first began to go above normal. Postmortem examination revealed, in addition to pulmonary tuberculosis, recent miliary seeding of liver, spleen, kidneys and pancreas. The source of the dissemination was identified as a small aneurysm in the thoracic aorta, the thrombus in the aneurysm being

partly caseous. Cultural results in this case indicated in the spleen 96,000 bacilli, in the liver 2,1000 bacilli, and in the kidney 9,800 tubercle bacilli per gram of tissue.

DISCUSSION

Several factors are involved in the localization and maintenance of tubercle bacilli in the various organs of the body. One thinks first of the richness of the arterial blood supply which, being much greater per gram of tissue in the spleen than the liver, is of undoubted importance in causing the greater localization of tubercle bacilli in the former organ. And those cases in which high bacterial counts in the liver were associated with intestinal tuberculosis further emphasize the importance of the blood supply—in this case the portal system. However, richness of blood supply cannot be the most important factor, for the kidney receives more arterial blood per gram than does the spleen. Moreover, as pointed out by Long and Faust (2), the adrenal gland, which has one of the richest blood supplies of any organ in the body, was found to be tuberculous in their series in only 16 per cent of the cases.

Probably more important than the amount of blood reaching an organ is the effectiveness of the organ as a filter of particulate material. In this respect the spleen is especially arranged to remove bacteria or other minute particles from the blood stream. In the kidney, as in the adrenals, it is probable that many bacilli are carried through the organ without being removed from the blood. It is possible, also, that tubercle bacilli may fail to remain in the kidney because of being eliminated in the urine, as shown by Yegian (5) in guinea pigs.

A third factor of importance in determining whether or not viable tubercle bacilli will be found in a given organ is the suitability of that organ as a nutrient medium for the microörganisms, or conversely, the capacity of the tissues of the organ for destroying the bacilli. In this respect Hirschberg and Arnold (6) have shown that tissue juice of the kidney has a greater inhibitory effect on the growth of tubercle bacilli than tissue juice from either the spleen or the liver, a fact which may be explained by the phosphatide splitting enzymes of the kidney (7). Certainly some factor must operate to protect the kidney from tuberculous infection, for Burn (8) has shown that postmortem cultures of kidney tissue for *pyogenic* bacteria are positive in 72 per cent of the cases while similar cultures of the spleen are positive in only 45 per cent of the cases.

The fact that tubercle bacilli can be recovered from the spleen in more than two-thirds of the patients dying from pulmonary tuberculosis indicates the frequency with which tubercle bacilli must gain access to the blood stream. On the other hand the fact that in 26 of our cases the indicated number of tubercle bacilli was 500 or less per gram of splenic tissue indicates that in many cases only very few bacilli enter the blood stream, and then only at rare intervals. This explains the known difficulty of recovering tubercle bacilli by blood culture when only 5 or 10 cc. of blood is drawn on a single occasion for cultural purposes.

SUMMARY

1. Human autopsy material has been cultured in order to determine the number of tubercle bacilli per gram of tissue in spleen, liver and kidney.

2. In a large majority of the cases cultures of the spleen were found positive for tubercle bacilli. A smaller proportion of liver cultures and even fewer of the kidney cultures were positive.

3. A rather close correlation was found between positive culture and histologically demonstrable tubercles in the same organ. A high bacterial count was associated with the type rather than the number of tubercles, the highest counts being found in those organs which had caseous, miliary tubercles.

4. The number of tubercle bacilli found per gram of splenic tissue was nearly always greater than the number of bacilli in liver and kidney. The only exceptions were cases with extensive tuberculous enteritis or other abdominal tuberculosis. In these cases the liver contained more tubercle bacilli than the spleen.

SUMARIO

1. A fin de determinar el número de bacilos tuberculosos por gramo de tejido esplénico, hepático, y renal, se hicieron cultivos de material humano obtenido en la autopsia.

2. En la gran mayoría de los casos, los cultivos del bazo resultaron positivos para el bacilo tuberculoso, pero la proporción fué menor para los cultivos hepáticos, y todavía menor para los renales.

3. Se encontró una relación bastante íntima entre la positividad de los cultivos y el hallazgo histológico de tuberculosis en el mismo órgano. Una elevada fórmula bacteriana se asoció con el tipo más bien que con la cantidad de tubérculos, encontrándose las numeraciones mayores en los órganos que mostraban tuberculosis miliares caseosas.

4. El número de bacilos tuberculosos descubiertos por gramo de tejido esplénico, fué casi siempre mayor que el encontrado en el hígado y riñón, correspondiendo las únicas excepciones a los casos en que había enteritis tuberculosa difusa u otra tuberculosis abdominal, en cuyos casos el hígado contenía más bacilos que el bazo.

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AN UNUSUAL MYCOBACTERIUM

Isolated from Sputum of a Man Suffering from Pulmonary Disease of Long Duration

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In human beings the aetiological relation of *Mycobacterium tuberculosis var. hominis*, and infrequently of *Mycobacterium tuberculosis var. bovis*, to pulmonary tuberculosis is so constant as ordinarily to preclude the consideration of other possible aetiological agents.[4] However there occur, as Baldwin has pointed out recently, instances in which there are signs suggesting acute pneumonic tuberculosis but in which tubercle bacilli cannot be demonstrated. In Baldwin's case, cardiospasm was associated with acute pneumonia and on several occasions a chromogenic avirulent acid-fast bacillus was isolated from the sputum. Baldwin showed that chromogenic acid-fast bacilli could be isolated from various fresh fruits and vegetables and pointed out the possibility that patients suffering from cardiospasm might draw into the respiratory tract regurgitated food containing nonpathogenic acid-fast bacilli. It is obvious that such microörganisms must be distinguished from tubercle bacilli.

In the case which is the subject of this report, the patient's illness was diagnosed as far advanced bilateral pulmonary silicotuberculosis; yet mammalian tubercle bacilli were never demonstrated. However, acid-fast bacillary forms were found intermittently in smears prepared from sputum and on several occasions cultures of an unusual mycobacterium were obtained. This report is concerned with the results of the study to determine the identity of this organism.

CLINICAL REPORT OF CASE

The patient was first seen in July, 1932. At that time his age was fifty-three years. He complained of cough, expectoration, pain in the chest, weakness, night sweats and indigestion and had lost 25 pounds (11.3 kg.). The patient connected the onset of these symptoms with an acute respiratory infection beginning about three months previously. He stated that he had been employed as an underground miner continuously for twenty-five years before the onset of his illness. His only known exposure to tuberculosis was casual contact with a neighbor in whom the disease was demonstrated later.

Examination of the chest showed diminished resonance, bronchovesicular breath sounds and a few fine râles over the upper third of each pulmonary field. A Mantoux test with Old Tuberculin, amount not recorded, was reported as giving uncertain positive results.

Roentgenographic examination of the chest showed thickening of the pleura on both sides with pleural calcification on the left and obliteration of both costophrenic angles.

[1] The Mayo Foundation, Rochester, Minnesota.
[2] Nopeming Sanatorium, Nopeming, Minnesota.
[3] University of Minnesota, Minneapolis, Minnesota.

[4] Those especially interested in atypical acid-fast bacteria derived from patients suffering from tuberculosis or from undiagnosed lesions suggestive of tuberculosis should consult the reports by Pinner

There were displacement of the trachea to the right and retraction of the hilar shadows toward the apex on both sides. The lower two-thirds of both pulmonary fields showed an increase of bronchovascular markings, rather extensive emphysema and uniformly scattered, rounded nodules 1 to 2 mm. in diameter of increased density. There was dense infiltration in the upper third of each pulmonary field with questionable cavitation. A diagnosis was made of far advanced, bilateral, pulmonary silicotuberculosis with secondary emphysema.

On July 14, 1932, the patient was admitted to the tuberculosis service of St. Luke's Hospital, Duluth, Minnesota, and shortly thereafter was transferred to Nopeming Sanatorium, where he was in the service of one of us (R. D.), who supplied the clinical report and invited another of us (W. H. F.) to undertake a study of the nature of the acid-fast bacilli found in specimens of sputum. Microscopical examination of smears of sputum failed to reveal acid-fast bacilli. The patient was treated by the usual sanatorium regimen without collapse therapy or medication. There was gradual symptomatic

TABLE 1

Results of intracutaneous tuberculin tests of the patient from whose sputum were obtained on several occasions acid-fast bacilli that were nonpathogenic for guinea pigs

DATE	MATERIAL	RESULT
1938	Old tuberculin (amount not recorded)	Questionably positive
November, 1941	0.00002 mg. PPD*	Negative
	0.005 mg. PPD	++
	0.01 mg. avian tuberculin	++
January, 1942	0.1 mg. Old Tuberculin	+++
	0.1 mg. extract from culture of acid-fast organism found in patient's sputum	++

* Purified protein derivative.

improvement but little or no change of objective findings. Four months after admission the patient was dismissed to his home.

Approximately six years later the patient was admitted to the tuberculosis service of St. Mary's Hospital, Duluth, Minnesota, because of an apparent increase of cavitation in both apices, as shown by a roentgenogram. The symptoms and physical findings were comparable to those recorded when the patient was seen first six years previously. Roentgenographic examination of the chest revealed further fibrosis and contraction in the upper third of each pulmonary field with an apparent increase of cavitation on both sides. Two of eight microscopical examinations of smears of sputum revealed acid-fast bacilli. About two months later the patient was transferred again to Nopeming Sanatorium. Treatment was essentially the same as on the previous admission. Because acid-fast bacilli were found repeatedly in sputum smears, the patient was considered a menace to public health and advised to remain at the Sanatorium. He was dismissed finally in the spring of 1941 after the acid-fast bacilli in his sputum proved to be nonpathogenic for guinea pigs.

There has been very little change of the patient's condition since his discharge. Be-

cause of dyspnoea he is unable to work except at occasional, very light jobs. However, he is able to live contentedly at home and to maintain a fair state of health. The patient's wife and six of his ten children were given a Mantoux test with 0.1 mg. of Old Tuberculin in 1938 and their reactions were all negative. Three of the children had repeated negative cutaneous reactions in 1940. The wife and seven of the ten children have had negative roentgenograms of the chest. Three of the children live in a distant city and have not been examined. Results of Mantoux tests given to the patient are shown in table 1.

ATTEMPTS TO DEMONSTRATE TUBERCLE BACILLI

During the past nine years a total of 91 sputum smears, stained by the Ziehl-Neelsen method, have been examined. We gratefully acknowledge the assistance of Dr. William Rademaker, Ely, Minnesota, in obtaining from the patient specimens of sputum. Fifty-one of the examinations were made of single specimens, while 40 represented sputum collected over a twenty-four-hour period. Acid-fast bacillary forms were demonstrated in 20 of 91 specimens. The smears that revealed acid-fast bacilli were made from specimens obtained during the period from March, 1938 to February, 1942, inclusive.

During the period from July, 1939 to November, 1941, inclusive, guinea pigs were inoculated intraperitoneally with 5 specimens of sputum and one specimen representing gastric lavage. All the specimens failed to produce signs of tuberculosis in the inoculated animals, although acid-fast bacilli were observed in smears from 2 of the specimens used to inoculate the animals.

Cultures: Because of the occasional finding of acid-fast bacillary forms in the sputum and the failure of lesions of tuberculosis to develop in guinea pigs inoculated intraperitoneally with sputum, specimens of sputum were cultured for acid-fast bacteria. Consequently specimens of sputum were sent to our laboratory in Rochester, Minnesota. A total of 6 specimens were received by one of us.

The first specimen was received July 3, 1941, the second and third specimens October 30, 1941, the fourth specimen November 3, 1941 and the fifth and sixth specimens September 9, 1942.

Portions of each specimen were treated with an equal amount of 5 per cent solution of oxalic acid according to the method suggested by Corper and Uyei. The precipitate obtained after centrifugation was spread over the surface of egg-yolk-agar media with and without glycerin[5] and incubated at 37.5°C. As will be mentioned later, guinea pigs were inoculated with portions of each of the respective specimens.[6]

From all specimens from which cultures were attempted, cultures of acid-fast bacillary forms were obtained. The physical characteristics of the colonies suggested bacteria other than *Mycobacterium tuberculosis var. hominis.* Consequently a somewhat detailed study was undertaken to establish the biochemical and pathogenic characteristics of each of the cultures obtained from the first 4 specimens. In the report that follows these cultures are referred to as cultures

[5] The preparation of this medium is described by Feldman.

[6] Specimens 5 and 6 were mixed inadvertently and the composite material was used for the making of cultures and the inoculation of guinea pigs.

1, 2, 3 and 4.[7] For the sake of brevity only data considered to be pertinent are included in this report.

CULTURAL AND MORPHOLOGICAL CHARACTERISTICS

The features of the colonies of the different strains of bacteria isolated were essentially similar in the original cultures and in subcultures on egg-yolk-agar media. Macroscopical colonies were usually evident within two weeks' time and occasionally as early as one week. In the early phases of growth the colonies varied up to 2 mm. in diameter and were circular in outline and similar in color to the medium, being pale yellow or gray. On further incubation at 37.5°C. a maximal diameter of colony of 3 or 4 mm. was attained. Isolated colonies were dome-shaped. The bacterial growth was smooth-surfaced and glistening. Culturally the vast majority of all cultures were nonchromogenic, being gray or faintly yellowish-gray. The growth in one of eight culture tubes inoculated with specimens 5 and 6 and an occasional other culture showed a golden yellow chromogenesis. Occasionally in original cultures there occurred colonies that had low centres and high peripheries, giving the colonies a "ring" appearance (figure 1). These colonies appear to be similar to the "ring" forms described by Meyn as occurring in, and being distinctive of, cultures of avian tubercle bacilli. The growth was butyrous in consistency and was easily miscible in physiological salt solution. A sweetish odor was produced in culture tubes.

Various media supported growth of the organisms. After several weeks on nutrient agar, colonies of the size of a pin-point developed at 37°C. Similar growth occurred on blood agar and starch agar. Neither haemolysis nor starch hydrolysis was demonstrated. A slight general turbidity with sedimentation occurred in the medium of Proskauer and Beck after several weeks. Growth was similarly slow in nutrient broth and in dextrose brain broth. In the latter medium the growth was limited to the upper 2 cm. of the medium and had a tendency to cling to the sides of the tube.

Microscopically, the bacteria were rod-shaped and pleomorphism was prominent. The majority of the cells were thin slender rods which seldom exceeded 2 μ in length; however, in culture 5, cells as long as 5 or 6 μ were common. The bacteria were acid-fast; the primary stain in some of the cells resisted decolorization with 2 per cent solution of hydrochloric acid in 80 per cent ethyl alcohol for twenty-four hours. In smears of the bacteria stained by the Ziehl-Neelsen method and by the Gram method, cells containing hyperchromatic granules were common. The bacilli were gram-positive.

[7] The culture obtained from the composite material representing sputum specimens 5 and 6 was isolated about ten months after the others were isolated and consequently was not available at the time cultures 1, 2, 3 and 4 were studied. Incomplete results following inoculation of guinea pigs, rabbits and chickens with a subculture of acid-fast bacilli isolated from sputum specimens 5 and 6 indicate that the bacilli were nonpathogenic for guinea pigs. At the time of writing the results of the pathogenicity tests for rabbits and chickens were not available. The bacilli sensitized the animals to avian tuberculin but not to mammalian tuberculin.

Physiological characteristics: The criteria proposed by Gordon for the classification of mycobacteria were selected for the study of the first four strains that were isolated from the patient. The optimal temperature for growth was found to be 37.5°C. and a minimum of growth occurred at 25° and at 47°C. The latter temperature permitted only a very scant growth. Viability of a portion of the cells was maintained after exposure to 60°C. for one hour.[8] Growth in litmus milk did not cause any change in its appearance. Cultures 1 and 2 reduced nitrates and utilized sorbitol, arabinose, trehalose, mannitol, levulose and sucrose as the sole source of carbon. Strains 3 and 4 did not reduce nitrates and in Merrill's basic medium they were able to use only trehalose and levulose as

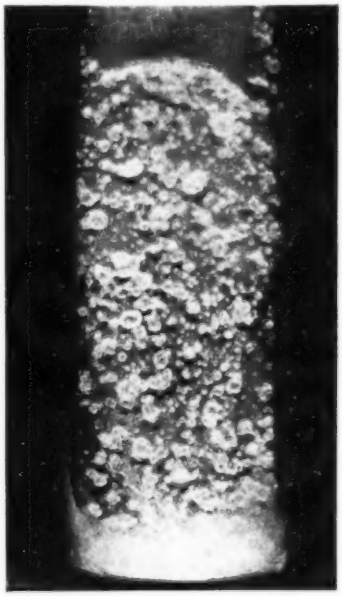

FIG. 1. Primary isolation culture. Egg medium seeded fourteen months previously with specimen 3 of sputum. Note numerous so-called ring colonies.

sole sources of carbon. None of the strains produced acid from any of the organic compounds just mentioned.

The results obtained from this portion of the study revealed that cultures 1 and 2 were similar to each other and that their features differed from those that were common to cultures 3 and 4. One may conclude, therefore, that the bacteria studied were dissimilar to the mycobacteria described by Gordon, by Gordon and Hagan, and by Karlson and Feldman.

AGGLUTINATION, CROSS-AGGLUTINATION AND AGGLUTININ ABSORPTION

The sera studied included (*1*) a specimen obtained from the patient in August, 1942, (*2*) a specimen of pooled serum from two rabbits which had received a

[8] Cells were suspended in sterile distilled water and comparable amounts of bacteria exposed to 60°C. in a water bath and tested for viability after variable periods from thirty minutes to a maximum of ninety minutes.

series of intravenous injections of living and heat-killed bacteria from culture 4 and (*3*) a specimen of serum from a naturally infected tuberculous chicken.

Concentrated or rapid plate antigens prepared and used as described in another communication from culture 4 and from a strain of *Mycobacterium avium* were employed for direct agglutination tests and for testing the specimens of serum after agglutinin absorption procedures. The bacteria used in the absorption procedures included one strain each of *Mycobacterium avium*, *Mycobacterium tuberculosis var. hominis*, the vole bacillus and two strains of *Mycobacterium tuberculosis var. bovis*. The recommendations of Krumwiede, Cooper and Provost for agglutinin absorption procedures were followed.

The agglutinative properties of the patient's serum were equally active for antigens prepared from culture 4 and from *Mycobacterium avium*. Complete agglutination of the cells in 0.03 cc. of both antigens was caused by 0.0025 cc. of the serum.[9] This agglutinative ability for culture 4 antigen was removed by agglutinin absorption procedures with cells of the homologous bacterial strain.

The agglutinin titres of the rabbit sera for culture 4 antigen was such that 0.0005 cc. of serum agglutinated 0.03 cc. of antigen. Agglutinin absorptions with the five cultures of acid-fast bacteria that were isolated from the patient showed that cultures 1 and 2 removed only 10 per cent of the agglutinins demonstrable by the antigen prepared from culture 4, whereas the other three strains removed all of the demonstrable agglutinins. Likewise, the antigen prepared from *Mycobacterium avium* removed all agglutinins for culture 4 antigen. No reduction of the concentration of these agglutinins was produced by exposure to cells of the human and bovine types of tubercle bacilli and of the vole bacillus.

The smallest quantities of the serum from a tuberculous chicken causing complete agglutination of 0.03 cc. of the *Mycobacterium avium* antigen and the acid-fast culture 4 antigen were 0.00125 and 0.0025 cc., respectively. Bacterial cells homologous to those from which the antigens were prepared removed all agglutinins that were demonstrable by either antigen.

The results of these studies are suggestive of the existence of a parasitic relation of culture 4 and cultures 3 and 5 to the patient from whom they were obtained and of the similarity of these strains to *Mycobacterium avium*. The lack of affinity of the cells from cultures 1 and 2 for the agglutinins produced in the rabbits against strain 4 is unexplained. Wilson has shown in studies on the classification of mycobacteria by agglutinin absorption that agglutinins produced in rabbits in response to strains of the major types of mycobacteria were absorbed more specifically by cells homologous to the major types. He showed also that *Mycobacterium avium* was able to absorb the greater part of the agglutinins from antiavium sera, but the human and bovine types, those affecting cold-blooded animals and other acid-fast types were not able to do so.

PATHOGENICITY

Guinea pigs: To determine the pathogenicity for guinea pigs of the various cultures isolated, 22 animals were inoculated subcutaneously. All 22 guinea

[9] Agglutination of the cells of culture 4 antigen has been observed with serum of some patients who had clinical tuberculosis, especially those whose tuberculosis was of long standing.

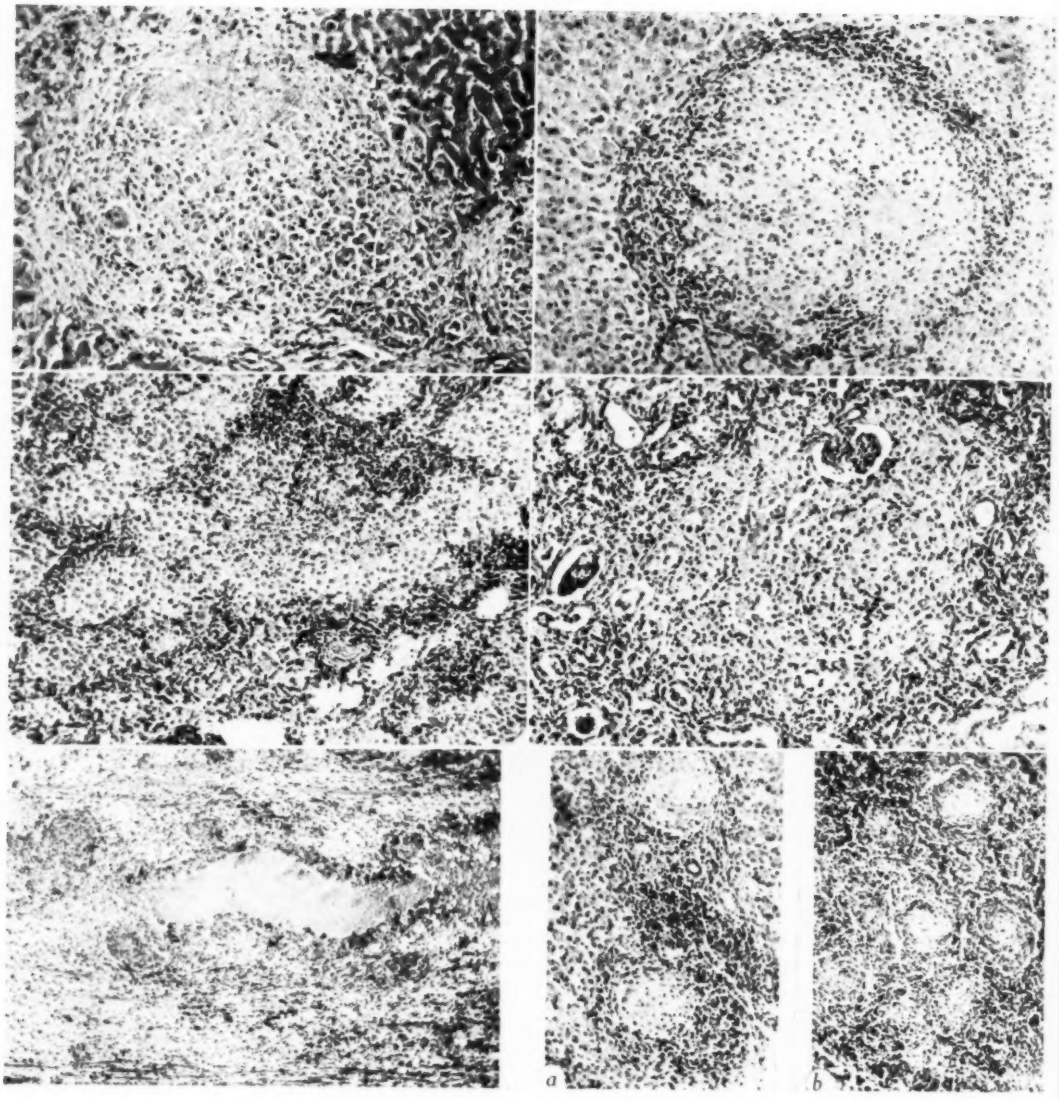

FIG. 2. (Upper left.) One of several interlobular epithelioid nodules with necrosis in the liver of a rabbit that died seventy-one days after intravenous inoculation with culture 1. Acid-fast bacilli present in the necrotic debris (× 67).

FIG. 3. (Upper right.) Nonprogressive epithelioid nodule with beginning central necrosis in the liver of a rabbit killed ninety-two days after intravenous inoculation with culture 2. Acid-fast bacilli present (× 87).

FIG. 4. (Center left.) Multiple epithelioid nodules with beginning necrosis in the lung of a rabbit killed ninety-two days after intravenous inoculation with culture 1. Acid-fast bacillary forms were numerous among the cellular constituents (× 73).

FIG. 5. (Center right.) Tuberculous lesion in the kidney of a rabbit inoculated intravenously seventy-one days before death with culture 1. The animal also had tuberculous lesions in the liver and lung (× 73).

FIG. 6. (Lower right.) (*a*) Two of many small, nonprogressive epithelioid nodules in the liver of a chicken killed ninety-two days after intravenous inoculation with culture 3 (× 100). (*b*) Numerous atrophic epithelioid nodules in the spleen of a chicken killed ninety-two days after intravenous inoculation with culture 3 (× 100). The lesions in both organs contained acid-fast bacilli.

FIG. 7. (Lower left.) Advancing, destructive tuberculosis-like process in the pectoral muscle of a chicken killed one hundred and sixteen days after intramuscular inoculation with culture 3. Acid-fast bacilli present. No parenchymal lesions (× 100).

pigs, except one, lived to be killed for necropsy sixty to seventy days after infection. The cultures proved nonpathogenic for guinea pigs. The results indicated that the bacteria were neither human nor bovine tubercle bacilli.

Rabbits: Each of the four cultures was used to inoculate 2 rabbits intravenously with 0.1 mg. Six of the rabbits were killed for necropsy ninety-two days after infection; one died after twenty-four days and one died after seventy-one days. Lesions definitely attributable to the inocula were found in each of the 8 rabbits. The lesions were not visible grossly. Microscopically, lesions were noted in the liver of 6 of the rabbits (figures 2 and 3); in the lungs of 4 (figure 4); in the spleen of 3 and in the kidney of one (figure 5). In no instance were lesions found in the bone marrow. At the time of necropsy attempts were made to recover by cultural methods acid-fast bacteria from different organs. The results were successful in 6 of the 8 animals.

The data regarding the pathogenicity of the respective cultures for rabbits showed that, with the exception of one rabbit that died twenty-four days after infection and in which an aggressive, destructive tuberculous process was present in the liver and in the spleen, the cultures did not appear to be highly pathogenic for rabbits.

Chickens: Sixteen chickens, previously negative to tuberculin, were used to test the pathogenicity of the four cultures. All were inoculated with 0.1 mg. of bacteria; each culture was used to inoculate 2 chickens intravenously and 2 chickens were inoculated intramuscularly into the pectoral muscles. One chicken died after forty-five days, 5 chickens were killed after ninety-two days, 2 were killed after one hundred and five days and 8 were killed after one hundred and sixteen days.

With one exception none of the chickens inoculated intravenously had signs of progressive tuberculosis at necropsy. A moderate number of small epithelioid ("hard") tubercles occurred in the spleen and liver of one of the 2 chickens inoculated intravenously with culture 3 (figure 6). In the one exception one of the 2 animals inoculated with culture 2 died in forty-five days with innumerable small epithelioid foci throughout the liver and spleen. Lesions also occurred in the bone marrow but the lungs were not affected. In some instances locally destructive tuberculous changes developed in chickens inoculated intramuscularly but in no instance was the intramuscular inoculation followed by subsequent involvement of the parenchymatous organs (figure 7).

COMPARISON OF PATHOGENICITY FOR CHICKENS OF CULTURES FROM THE PATIENT AND A STRAIN OF VIRULENT AVIAN TUBERCLE BACILLI

Two chickens, negative to tuberculin, were inoculated intramuscularly with 0.1 mg. of a strain of tubercle bacilli isolated one month previously from the tuberculous spleen of a chicken that had died with naturally acquired tuberculosis. The chickens died seventy-three and eighty days respectively after inoculation. Each had large caseous abscesses in the muscle at the site of inoculation and striking microscopical tuberculous changes in the liver and spleen. The lungs were not affected.

Compared with the results following intramuscular inoculation of the cultures from the patient, the results with the virulent avian tubercle bacillus were impressively more striking. The cultures from the patient, while inducing in some chickens a progressively destructive process in the muscles at the site of inoculation, remained more or less localized. In no instances were the lesions in the muscles followed by a dissemination of the disease to the liver or spleen. The avian tubercle bacillus not only incited a marked tuberculous disease in the muscles but in addition involved the spleen and liver in a fashion typical of severe tuberculosis in these organs.

Another difference of some interest was the tremendous number of acid-fast bacilli in the lesions produced by the organism of avian tuberculosis. Acid-fast bacteria were present in the lesions produced by the cultures from the patient but were markedly fewer than in the lesions produced by the avian tubercle bacilli.

These observations showed that, although capable in some instances of inducing a locally destructive lesion, the cultures from the patient did not have sufficient virulence for the chickens inoculated intramuscularly to prosper in these birds and establish a progressively fatal disease in such organs as the liver and the spleen.

SENSITIVITY TO TUBERCULIN

With a few exceptions the animals inoculated with the cultures from the patient and living after thirty days were tested with tuberculins. The products used were avian tuberculin, kindly supplied by the Bureau of Animal Industry, United States Department of Agriculture; Gilliland's Old Tuberculin, diluted 1:100, and a tuberculin-like product prepared by one of us (W. A.) from culture 1.[10] In all instances the intracutaneous test was used. The amount of tuberculin injected was approximately 0.02 to 0.03 cc. In no instance was the animal inoculated with more than two tuberculins at any one test period. Most of the animals were tested with one or more of the tuberculins on at least two different occasions. Subsequent tests were applied at least thirty days after the previous tests. The results were recorded forty-eight hours after the tuberculins were injected.

It was found that all of the cultures sensitized guinea pigs, rabbits and chickens to avian tuberculin and less consistently to mammalian tuberculin. Positive reactions to the "tuberculin" prepared from culture 1 were elicited less frequently than was true of avian tuberculin.

The most interesting information obtained from these results was the greater ability of all of the cultures from the patient to sensitize guinea pigs, rabbits and chickens to avian tuberculin than to mammalian tuberculin or to the product prepared from culture 1. In guinea pigs the presence of marked sensitivity to avian tuberculin in animals the great majority of which did not have any demonstrable foci of infection at the time they were killed, is worthy of note. The

[10] The product was prepared on synthetic medium according to the method of Dorset and Henley. The final product obtained was of the same relative strength as avian tuberculin which contains 25 per cent Old Tuberculin.

significance of this is not apparent. It is likely, however, that the sensitivity developed at the same time as, and in association with, foci that were established at the site of inoculation soon after the introduction of the bacteria. The marked resistance of the guinea pig to the cultures from the patient would account for the fact that the initial lesions eventually disappeared. Perhaps if the animals had been kept under observation for a longer period the sensitivity to tuberculin would have diminished gradually so as to be no longer detectable.

Why so few of the guinea pigs reacted to the "tuberculin" prepared from culture 1 is unexplained.

In the rabbits and chickens the few negative reactions recorded for avian tuberculin occurred for the most part in animals in which lesions were not found. One notable exception was the chicken that died forty-five days after intravenous inoculation with culture 2. When tested thirty-three days after infection, this bird gave negative results with avian tuberculin and with "tuberculin" prepared from culture 1. As mentioned previously, this bird at death was found microscopically to have a striking tuberculosis-like involvement of the liver and spleen with numerous foci in the bone marrow.

SUMMARY AND CONCLUSIONS

From the results of the studies recounted certain facts have been established. These are:

1. From the sputum of a male patient in the sixth decade of life, who for twenty-five years prior to the onset of his illness had been an underground miner, acid-fast bacilli dissimilar to *Mycobacterium tuberculosis var. hominis* or *var. bovis* were cultured on several occasions. Although roentgenographic examination of the chest appeared to justify a diagnosis of far advanced, bilateral pulmonary silicotuberculosis, specimens of sputum containing acid-fast bacillary forms failed repeatedly to produce tuberculosis in guinea pigs. The patient's wife and six of his ten children failed to react to tuberculin and his wife and seven of the children available for examination had normal roentgenograms of the chest.

2. The cultures obtained from specimens of sputum sensitized guinea pigs, rabbits and chickens to avian tuberculin and less consistently to mammalian tuberculin.

3. None of the cultures were pathogenic for guinea pigs although the cultures did have a relatively low but definite pathogenicity for rabbits. With one exception, all chickens inoculated intravenously with the respective cultures were without signs of progressive tuberculosis at necropsy. Intramuscular inoculation of chickens resulted in some instances in the development of a destructive tuberculosis-like process without extension to the parenchymal organs.

4. The resistance of the respective cultures to oxalic acid, their cultural and tinctorial characteristics, their ability to induce sensitivity to tuberculin, their agglutinogenic features and their ability to establish limited tuberculosis-like lesions in rabbits and chickens all indicate a bacterium closely related to true tubercle bacilli. Although having many features suggestive of avian tubercle bacilli, the cultures studied were not typical of the organism of avian tuberculosis.

5. On the basis of certain biochemical and agglutinogenic characteristics the cultures were not all alike. This suggests an instability of the mycobacteria present in the pulmonary lesions although the specific mechanism is unexplained.

6. Conceivably the silicosis of the patient may have provided a favorable threshold for the parasitism of an organism ordinarily nonpathogenic for human beings.

7. The results of the study suggest that patients suffering from tuberculosis-like disease from which typical forms of tubercle bacilli cannot be demonstrated should be subjected to comprehensive studies to demonstrate the possible existence of atypical or unusual strains of mycobacteria.

SUMARIO Y CONCLUSIONES

Por los resultados de los estudios descritos se han establecido ciertos hechos, a saber:

1. En varias ocasiones se cultivaron, del esputo de un enfermo varón en el sexto decenio de la vida que por 25 años antes de iniciarse su enfermedad había sido un minero subterráneo, bacilos ácidorresistentes distintos del *Mycobacterium tuberculosis var. hominis* o *var. bovis*. Aunque el examen roentgenográfico del tórax parecía justificar un diagnóstico de silicotuberculosis pulmonar bilateral muy avanzada, las muestras de esputo que contenían formas bacilares ácidorresistentes dejaron repetidamente de producir tuberculosis en los cobayos. La esposa del enfermo y seis de los 10 hijos tampoco reaccionaron a la tuberculina, y la esposa y siete hijos disponibles para examen mostraron roentgenogramas torácicos normales.

2. Los cultivos obtenidos de muestras del esputo sensibilizaron a los cobayos, conejos y pollos a la tuberculina aviaria y menos constantemente a la del mamífero.

3. Ninguno de los cultivos resultó patógeno para el cobayo, aunque en general mostraran una patogenicidad relativamente baja pero bien definida para los conejos. Con una sola excepción los pollos inoculados intravenosamente con los cultivos respectivos no mostraron en la autopsia signos de tuberculosis evolutiva. La inoculación intramuscular de los pollos produjo en algunos casos un proceso tuberculoideo destructor que no se extendió a los órganos del parénquima.

4. La resistencia de los cultivos respectivos al ácido oxálico, sus características culturales y tintóreas, su capacidad para provocar sensibilidad a la tuberculina, sus características aglutinógenas, y su capacidad para establecer lesiones tuberculoideas limitadas en los conejos y pollos, todo ello indica que se trata de una bacteria íntimamente relacionada con los verdaderos bacilos tuberculosos. Aunque poseían muchas características indicativas de bacilos tuberculosos aviarios, los cultivos estudiados no fueron típicos de dichos microbios.

5. Tomando por base ciertas características bioquímicas y aglutinógenas no todos los cultivos eran semejantes, lo cual indica una inestabilidad de las micobacterias presentes en las lesiones pulmonares aunque no se ha explicado el mecanismo específico.

6. Cabe concebir que la silicosis del enfermo pudo haber preparado el terreno

para el parasitismo de un microbio que ordinariamente no es patógeno para el hombre.

7. Los resultados del estudio indican que siempre que un enfermo padezca de una afección tuberculoidea, en la cual no pueden encontrarse formas típicas de los bacilos tuberculosos, debe ser objeto de estudios minuciosos para demostrar la posible existencia de cepas atípicas o extrañas de micobacterias.

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TREATMENT OF TUBERCULOUS TRACHEOBRONCHITIS[1]

ROBERTS DAVIES

In the past ten years a great deal has been published on the subject of tuberculous tracheobronchitis. The importance of the subject is now rather generally appreciated. Something is known of the pathogenesis of the condition; the symptoms, physical and X-ray findings and sequelae have been well described. However, the questions of prognosis and proper treatment of this important complication of pulmonary tuberculosis are by no means settled.

Reported mortality varies from no deaths in a small series reported by Sandler (44) to 50 per cent of a larger group reported by Pierson and Samson (39). Although there are some notable exceptions (11, 33, 50), the majority of authors feel that the outlook is very poor, with from 20 per cent to 50 per cent deaths to be expected in two to four years and very few complete recoveries (2, 8, 28, 34, 39, 48).

There is a tremendous variation in the types of treatment recommended in the literature. General sanatorium care and symptomatic treatment have received little emphasis and are apparently generally considered to be of slight importance.

The indications for collapse therapy in pulmonary tuberculosis complicated by tuberculous bronchitis have been clarified in some recent publications. It is obvious that collapse is frequently necessary to control the parenchymal lesions and that it may be useful in the prevention and possibly in the treatment of the dangerous sequelae of bronchial obstruction, namely bronchiectasis and pulmonary suppuration. Chamberlain and Gordon (6) have pointed out that collapse measures should be a step ahead of progressive bronchial obstruction in order to forestall such complications. However, the sequence in which collapse procedures are ordinarily used in uncomplicated pulmonary disease has apparently produced unfortunate results when applied to cases complicated by bronchial obstruction. Almost all authors uniformly condemn the use of phrenic nerve operations in such cases because of increased difficulty in evacuating secretions and increased danger of atelectasis (47, 48). The use of pneumothorax, ordinarily considered the simplest and safest of all collapse procedures, also appears hazardous. A high mortality (Tuttle *et al.* (48)—38 per cent; Chamberlain and Gordon (6)—33 per cent) and a very high incidence of such unfortunate complications as empyema, bronchopleural fistula and unexpansile lungs have been reported (4, 6, 11, 47). It is apparent that, even with a satisfactory result and with thoroughly healed bronchial tuberculosis, pneumothorax cannot be considered a reversible or temporary procedure since reëxpansion may result in a fulminating reactivation of both bronchial and parenchymal disease (1, 6). Many writers feel that thoracoplasty is the best procedure for most cases of pulmonary disease complicated by bronchial tuberculosis when collapse is necessary (1, 6, 35, 47, 48). Several authors have pointed out that bronchial

[1] From Nopeming Sanatorium, Nopeming, Minnesota.

lesions should be healed or at least not progressive before thoracoplasty can be undertaken (8, 20, 35, 39). Alexander and his colleagues (1) advise that, whenever the main bronchus is involved, the thoracoplasty must be complete even if only one lobe is diseased, since later emphysema, atelectasis or bronchiectasis are very likely to involve the uncollapsed portion of the lung. Open cavity drainage may be done if cavitation persists under a complete thoracoplasty (1, 6, 43).

Pneumonectomy and lobectomy have also been used in the treatment of pulmonary disease distal to a tuberculous bronchial stenosis (1, 6, 7, 9, 14, 40, 43, 48). In general the mortality from pneumonectomy in such cases has been 40 per cent and from lobectomy 20 per cent. Alexander and his co-workers (1) believe the indication for pneumonectomy is a tight, undilatable bronchial stricture with severe symptoms from retained secretions. These authors and Chamberlain and Gordon (6) emphasize that before pneumonectomy can be done the bronchial lesion must be healed and the contralateral lung must be free of any active disease. Dolley and Jones (14) believe that thoracoplasty before pneumonectomy will reduce the mortality, but Alexander and his colleagues (1) feel that such patients are too sick to stand both thoracoplasty and pneumonectomy and that thoracoplasty may cause death by making a partial obstruction complete.

Several methods of direct attack upon the tracheal or bronchial lesions have been described. Bronchoscopic removal of granulations and granulomata (31), dilatation of obstructions with aspiration of retained secretions (3, 7, 11, 20, 22, 23, 24, 25, 28, 42), ultraviolet irradiation applied locally through a bronchoscope (23, 24, 25), X-ray therapy directed to the bronchial lesion (12, 39, 41, 42), electrocautery (13, 16, 20, 23, 24, 25, 34, 36, 39, 42, 50), and cautery with silver nitrate solutions (4, 5, 6, 7, 11, 16, 20, 22, 23, 24, 25, 26, 28, 34, 43, 45, 48, 50) varying from 5 per cent to 50 per cent have all been advocated. Removal of granulations and especially of granulomatous tumors usually followed by cautery of the base has been practiced by many workers (5). It has been condemned by others because of the danger of bronchogenic dissemination of disease from aspiration of bacilli-laden debris and because, in the case of tuberculomata, many of which are not ulcerated, it creates a new ulcerated area (7). Dilatation of obstructions and aspiration of secretions is generally conceded to be useful in relieving symptoms but is usually felt to be only palliative and of no great permanent value (11, 31). Kernan (23, 24, 25) feels that more permanent results can be achieved by dilatation and ionization of the strictured segment with a special copper electrode, and that direct ultraviolet irradiation is effective in healing active lesions and produces less scar than does treatment with any form of cautery. X-ray therapy has been advised by Pierson and Samson (39) for chronic lesions which cannot be treated by cauterization because of technical difficulties. They report good results in 6 out of 12 cases. Davenport (12) reports a series of 7 cases treated by X-ray therapy with one death. The other 6 cases were improved but none were cured.

About two-thirds of the authors who have considered the use of electrocautery

or silver nitrate in the treatment of tuberculous tracheobronchial lesions favor such cauterization (4, 6, 11, 16, 21, 22, 26, 28, 36, 39, 42, 43, 45, 48, 50). Most of those who oppose such local treatment have apparently either not used it at all or have used it with intervals of a month or longer between treatments. Some oppose it as being illogical. They feel that tuberculosis involves the whole thickness of the bronchial wall in most cases and that it is unreasonable to treat only the surface of such a deep-seated lesion (8). They also feel that in most cases the involvement extends deep into the finer ramifications of the bronchial tree, beyond the reach of the bronchoscope, and that treatment of the visible lesion only can be of little value (12, 32, 40).

In general those who have used local cautery have presented the most favorable results. In a series of 189 cases collected from the literature that were treated either with electrocautery or silver nitrate, 76 cases, or 40 per cent, were reported as "healed or well" and 28 cases, or 15 per cent, were reported as dead (11, 13, 36, 45, 48, 50). In a similarly collected series of 121 cases who received no local cautery or cautery only at long intervals, 13, or 11 per cent, were reported "healed or well" and 31, or 26 per cent, were dead (2, 5, 8, 11, 12, 41). It should be remembered that such results cannot be taken at face value since it is not possible to ascertain from the published data if the two series are comparable from the standpoint of age, severity of the parenchymal lesion, length of observation and criteria of healing or cure.

MATERIAL

The basis of this report is a study of all cases of tuberculosis with bronchoscopically demonstrable ulceration or significant obstruction, that have been observed since the treatment of such lesions was first undertaken at Nopeming Sanatorium on November 24, 1937. Patients have not been bronchoscoped routinely but have been selected for bronchoscopy because of clinical or X-ray evidence of a tracheal or bronchial lesion. One hundred and fifty-six patients were bronchoscoped out of 923 inpatients treated at the Sanatorium during the period of this study. Because of the method of selection some cases may have been missed, but no patients have come to autopsy with previously unrecognized bronchial tuberculosis. Sixty-four per cent of all patients dying at the Sanatorium in the period covered by this report have been autopsied.

This series consists of 26 patients, 3 male and 23 female, all belonging to the white race. Their ages at diagnosis of the bronchial lesion varied from twenty to fifty years, with 11 each in the third and fourth decades, 3 in the fifth decade and one in the sixth decade.

As judged from the history and a review of all available records and X-ray films, the time interval between the onset of tracheal or bronchial tuberculosis and the date of diagnosis of such lesions by bronchoscopy varied from less than one month to more than eight years. In 10 cases this interval was less than four months, in 3 additional cases, less than one year and in the remaining 13 cases, more than one year.

The pulmonary lesions present at the time of bronchoscopic diagnosis were minimal in one case, moderately advanced in 11 and far advanced in 14. The

pulmonary lesions were bilateral in 17 cases and unilateral in 9. Only 2 cases showed definite X-ray evidence of cavitation at the time the bronchoscopic diagnosis was made although others had previously shown definite cavities. X-ray evidence of atelectasis was present in 22 cases and in one other case atelectasis developed later. Sixteen patients had a pneumothorax. In 3 cases the collapse was bilateral and in 3 cases it was extrapleural. Four patients had a unilateral thoracoplasty. All but one patient had tubercle bacilli in the sputum on microscopical examination, and that one case had had positive sputum previously.

Symptoms varied greatly in severity. One patient had no symptoms whatever and several were extremely ill with persistent and harassing cough, constant wheezing, severe dyspnoea, chest pain and high fever. In general, symptoms were very severe in 9 patients, slight to moderate in 17 and absent in one. Twenty-one patients had a wheeze, 15 complained of chest pain and 12 had a severe and troublesome cough.

Only those patients who showed definite ulceration or significant bronchial obstruction are included in this series. Patients showing only oedema or submucous infiltration were not included because we felt that at least some of such lesions are probably nonspecific, and at any rate are mostly of minor clinical importance if they do not produce obstruction or progress to ulceration. Tuberculomata and mucosal or submucosal tubercles without ulceration or obstruction were also excluded as being relatively unimportant. Such lesions, of course, should be followed by repeated bronchoscopies until healing has taken place.

In 12 cases the bronchial involvement was on the right side and in 15 cases on the left side. In 4 cases the trachea was affected. Fifteen patients had tuberculosis of a main bronchus and in one of these the involvement was bilateral.

Definite ulceration was seen in 19 of the 26 cases. In 8 cases the lesion was 3 cm. or more in length when first diagnosed. In 7 patients the lumen of a main bronchus was reduced to 3 mm. in diameter or less and in 9 patients the diameter of a lobar bronchial orifice was reduced to 2 mm. or less. Completely encircling ulceration of a main bronchus was seen in 4 cases at the original bronchoscopy and a similar lesion of a lobar bronchus in 2. Encircling lesions without encircling ulceration were found in a main bronchus in 4 cases and in a lobar bronchus in 7 cases at the time of diagnosis.

TREATMENT

General: Complete bed-rest, preferably in a sanatorium, with provision of a diet adequate in all essentials has long been recognized as the basis of treatment for tuberculosis of any organ except possibly the skin. There seems to be no reason why the tracheobronchial tree should be an exception. Regardless of the possible inactivity of parenchymal lesions, our patients have been kept on strict bed-rest without bathroom privileges as long as there has been any evidence of active bronchial disease and usually for several months after such evidence is last seen. In the very few cases where for one reason or another this program was not followed, results have been less satisfactory than would otherwise have been anticipated.

Ascorbic acid has been advised for the treatment of mucosal tuberculosis in

general, but we have had no experience with its use in bronchial lesions (43). General ultraviolet irradiation was used in only one patient and then because of an associated intestinal tuberculosis. No effect on the tracheobronchial lesion was noted.

Symptomatic: The more severe symptoms of bronchial tuberculosis are so distressing as to demand relief if relief is possible. It seems almost axiomatic that relief of a continuous, harassing cough with nausea, vomiting and sleeplessness will improve the patient's general condition, decrease local irritation and promote healing of the bronchial lesion. We have found the following regimen to be effective, and often dramatically effective, in relieving such severe symptoms:

1: Room temperature of 75°F. or over with all windows closed in cool or dry weather.
2: Continuous steam from an inhalator placed at the head of the bed.
3: Minimum fluid intake of 3,000 cc. in twenty-four hours.
4: Postural drainage two to four times daily, especially if a main bronchus or lower lobe bronchus is obstructed.
5: Expectorants, usually syrup of ipecac, 5 to 10 minims, three or four times daily.
6: Antispasmodics, usually ephedrine, $\frac{3}{16}$ to $\frac{3}{4}$ grain, three or four times daily.
7: Sedatives, usually phenobarbital, $\frac{1}{2}$ grain, three or four times daily.
8: Atropine and opium derivatives, including codeine, are contraindicated because they interfere with expectoration by making sputum more viscid, in the case of atropine, and by diminishing the cough reflex, in the case of opium products.

Collapse: In 22 of our 26 cases some major form of collapse therapy, either intrapleural pneumothorax, extrapleural pneumothorax or thoracoplasty was in use during the period when active tuberculous bronchitis was present. Intrapleural pneumothorax was used in 15 instances, extrapleural pneumothorax in 3 and thoracoplasty in 7. In 3 of the thoracoplasty cases, pneumothorax had been used previously. Because of the small size of the series and especially because there is no adequate control series in which collapse was not used, we can draw no reliable conclusions as to the advantage of collapse therapy for the patient with tuberculous bronchitis.

However, some observations regarding the complications of pneumothorax in patients with tuberculous bronchial disease seem worth while. We are unable to confirm the unusually high incidence of empyema (30 per cent to 40 per cent) reported by others (6, 48). Empyema occurred in one of the 3 cases of extrapleural pneumothorax, and in that case was cured quickly by aspiration and oleothorax. It occurred only twice among the 15 patients with intrapleural pneumothorax. In one of these cases the empyema followed an unsuccessful attempt at pneumonectomy and in the other was present when the patient was first admitted to Nopeming Sanatorium from another institution.

On the other hand, the high incidence of unexpansile lungs is amply confirmed by our experience, since 7 of our 15 cases of intrapleural pneumothorax are so classified (4, 6, 11, 47). In 2 cases attempted reëxpansion resulted in a severe and acute recurrence of a previously healed bronchial lesion. In only a single case was reëxpansion successful and uneventful.

Realizing that accumulating experience may change our attitude considerably, we have tentatively adopted the following indications for the use of collapse therapy in patients with bronchial tuberculosis. If the parenchymal lesion would indicate collapse were no bronchial lesion present, collapse should be considered. However, if the parenchymal lesion is not in itself imminently dangerous and if its course is not unfavorable, and if the bronchial lesion does not produce significant obstruction, one should give strict bed-rest without collapse therapy a longer trial before initiating collapse than would be done if no bronchial lesion were present. If the bronchial lesion reduces the lumen of a main bronchus to 3 or 4 mm. in diameter or less, or reduces the lumen of a middle or lower lobe bronchus to 2 or 3 mm. or less, and if the obstruction cannot be rather promptly relieved, collapse is urgently indicated. The purpose of collapse is to prevent or at least minimize the development of bronchiectasis, which is likely to be tuberculous in nature and is a dangerous complication and difficult to handle (1, 6, 8, 9, 14, 35, 40, 48). Equal stenosis of an upper lobe bronchus alone is somewhat less serious, because drainage is more adequate, bronchiectasis does not develop so readily and is less likely to be disabling if it does develop.

If collapse therapy is indicated, thoracoplasty is the procedure of choice, provided significant obstruction of a main bronchus or a lobar bronchus is present or seems likely to develop (1, 6, 35, 47, 48). In such cases there is very little chance of successful reëxpansion of the lung after pneumothorax, and by doing thoracoplasty first the possibility of empyema is largely avoided. If thoracoplasty is done with a free intrapleural space, the surgeon should be alert for the development of a postoperative intrapleural effusion with fever and respiratory distress, which can be relieved by aspiration. In general, when thoracoplasty is done it should be complete (1). In a few cases in which the bronchial lesion is limited to an upper lobe bronchus or in which a lesion of the main bronchus has healed completely and without obstruction and in which the lower lobe is entirely free from tuberculosis, bronchiectasis or emphysema, a partial thoracoplasty may be sufficient. If the indications for collapse are not urgent, the bronchial lesions should be healed before thoracoplasty is done (8, 20, 35, 39).

Pneumothorax should be reserved for those cases in which the parenchymal lesion demands collapse and in which the bronchial lesion is mild and does not produce significant obstruction. Pneumothorax may also be useful occasionally as a temporary procedure for a patient who presents urgent indications for collapse but seems unable to stand thoracoplasty. Such cases are likely to be infrequent.

Phrenic nerve operations should not be used for patients with bronchial tuberculosis (47, 48). The interference of diaphragmatic paralysis with the processes that keep the bronchial tree cleansed of secretions is out of proportion to its largely unpredictable effect on parenchymal lesions.

If the patient has a tight, undilatable stricture of a lobar or main bronchus with toxaemia from retained secretions, thoracoplasty is likely to be dangerous because the added collapse may make the bronchial obstruction complete (1). In such a case lobectomy should be done if a lobar bronchus is involved and

pneumonectomy if a main bronchus is involved, providing that the contralateral lung and, in the case of lobectomy, the other homolateral lobe or lobes are entirely free of active disease and the bronchial lesion is healed. It is preferable that the bronchial lesion shall have been healed for a period of a year or more, since active disease may persist in the bronchial wall long after mucosal ulcerations have disappeared. If lobectomy is done on a patient with apparently healed tuberculosis in another lobe of the same lung, the lobectomy should be either preceded or followed by thoracoplasty to prevent reactivation of the disease by compensatory expansion of the remaining lobe. Likewise undue expansion of the contralateral lung after pneumonectomy must be prevented (14).

A persistent cavity under a complete thoracoplasty may be treated by open drainage (1, 6, 43). Such a procedure, as illustrated by one of our cases, may permit healing of a bronchial tuberculosis which otherwise resists all treatment.

Local treatment: Since the treatment of tuberculous lesions of the trachea and bronchi by the application of cautery through the bronchoscope has been vigorously condemned by some authors as being useless and even harmful (15, 20, 31, 32, 33, 46, 49), it seems worth while to make some statement as to our understanding of the rationale of such procedure. The opponents of local treatment feel that it is useless to treat only the surface of a lesion which is often deep-seated in the bronchial wall (8) or to treat only the visible portion of a lesion which may extend into inaccessible bronchial divisions (12, 32, 40). Some authors feel that local treatment, especially with electrocautery and the greater concentrations (20 per cent to 30 per cent) of silver nitrate, causes excessive scarring and stenosis (7, 24) and that even bronchoscopy without treatment may be harmful by increasing local irritation (12).

The aim of local treatment is two-fold: first, to promote the healing of ulcerations in the tracheobronchial tree and, second, to prevent or minimize stenosis. Condon (10) has shown that the extent and rapidity of healing of ulcers produced surgically in the tracheas of rats is inversely proportional to the amount of inflammatory reaction in the base of the ulcer. We believe that silver nitrate cautery aids in the healing of tuberculous ulcers in the human bronchi and trachea by preparing the ulcer base to receive and support the ingrowing epithelium. This perhaps may be accomplished by reducing granulations and combating secondary infection. We do not believe it has anything to do with the tuberculous nature of the lesion. Since silver nitrate has been widely and successfully used for many years to promote healing of dirty, granulating, superficial ulcerations in other parts of the body, it is not surprising that it should be effective in the treatment of similar lesions in the tracheobronchial tree.

The epithelization of tracheobronchial ulcers is important in that it removes one and often the only source of bacilli in the sputum, thus preventing bronchogenic dissemination of tuberculosis and further bronchial or tracheal infection by implantation. It is also evident that epithelization will prevent a further increase in the size and extent of the ulcer. This may be very important in preventing a localized ulcer from eventually involving the whole circumference of the bronchial lumen. In the bronchus, as in other hollow organs, circumfer-

ential ulceration is likely to be followed by very serious stenosis, because of the concentric contraction of the fibrous tissue that is laid down as part of the repair process. Hilding (17, 18, 19) has shown in animals that, in a rigid walled cavity with an epithelial lining such as the paranasal sinuses, circumferential ulceration will, in healing, produce a membranous type of obstruction which may be almost complete. We and others have observed the same process in the bronchus with its semirigid walls (9, 13, 36, 48, 50). Such obstructions are serious and very difficult to handle. The epithelization of a bronchial ulcer can be expected to favor the healing of the tuberculous lesion in the deeper portions of the bronchial wall by preventing secondary infection and superinfection from bacilli-laden sputum. It will also minimize the mechanical trauma inflicted by respiratory motions, especially with severe cough, when the opposing swollen bronchial surfaces may come in contact with each other.

It is unfortunately true that in many cases a portion of the lesion is not accessible for treatment and this fact no doubt accounts for some failures. However, as the proximal portion of an extensive lesion heals, more and more of the distal portion in the main bronchus and often in lobar bronchi can be reached and treated. Lesions in wholly inaccessible intralobar branches are, of course, considerably smaller than comparable lesions in a main bronchus because of the small size of the bronchial tube involved and may therefore be expected to heal somewhat more readily. Healing with considerably stenosis is also a much less serious matter in a small intralobar branch bronchus than in a main bronchus because of the much smaller volume of lung affected.

It seems reasonable to assume that the chief factor determining the amount of scar tissue laid down in the bronchial wall during the healing of a bronchial lesion will be the amount of normal tissue that has been destroyed. It seems likely that electrocoagulation, especially if applied for an unduly long time, may destroy normal tissue below the layer of tuberculous granulation tissue in the base of an ulcer. Several authors feel that such excessive destruction does actually take place and that treatment by electrocoagulation does cause excessive scar (5, 11). The same objection does not necessarily apply to a chemical cautery since in this case, unless the agent is so powerful as to completely destroy coagulated tissue (which is not true of silver nitrate), the destruction is limited to the thin layer of tissue which is coagulated immediately upon application of the chemical and which then acts as a barrier to further tissue destruction. It is evidently the impression of most authors that silver nitrate solution in strengths up to 35 per cent does not cause more scar than would be expected from the natural healing of the lesions, and in our own cases we have seen no evidence of a deleterious effect (6, 11, 22, 26, 28, 43, 45, 48, 50).

The harmlessness of bronchoscopy itself for the tuberculous patient with or without tracheobronchial lesions, providing that proper technique is used and that the contraindications are respected, has been attested by reports of numerous investigators (1, 5, 6, 7, 8, 9, 11, 16, 23, 24, 25, 27, 29, 30, 31, 32, 33, 38, 39, 40, 41, 42, 44, 45, 48, 50). In a series of approximately 400 bronchoscopies on 156 patients we have not seen evidence of harm in a single case.

We have respected the following relative contraindications to bronchoscopy in the tuberculous:

1: Severe tuberculous laryngitis, especially if the lesion is acute and more especially if there is any narrowing of the airway.
2: Acute respiratory infection.
3: Recent frank pulmonary haemorrhage.
4: Severe illness or hopeless prognosis whether from tuberculous or nontuberculous disease if bronchoscopy cannot be expected to be important either diagnostically or therapeutically.

Technique: Because we believe that technique is of considerable importance in the treatment of tuberculous bronchial lesions, we shall outline our procedure in some detail. Before bronchoscopy the patient's meal is withheld and he is given some sedation, usually $1\frac{1}{2}$ grains of sodium pentobarbital and $\frac{1}{6}$ to $\frac{1}{4}$ grain of morphine sulfate one hour preoperatively. Considerable effort is made in psychological preparation of the patient so that he comes to the operating room with a minimum of fear. Local anaesthesia is used with a freshly prepared solution of 5 per cent cocaine and 1:2,000 adrenalin being applied very thoroughly but gently to the pharynx, pyriform sinuses, epiglottis and larynx. One cubic centimeter of the same solution is slowly dropped into the larynx. The bronchoscopy is done with the usual aseptic surgical technique and as gently as possible. A bronchoscope larger than 7 mm. is never used and in small patients or in those with narrowed main bronchi, a 5 mm. size is used. The bronchoscope is often introduced without the aid of a laryngoscope since with many individuals this procedure is easier both for the patient and for the operator. If necessary to relieve cough, the cocaine-adrenalin mixture is sprayed through the bronchoscope with a bronchoscopic atomizer. The bronchoscope should never be passed over an active bronchial lesion. By careful positioning of the patient's head it is often possible to pass the bronchoscope beyond a localized tracheal tuberculosis without traumatizing the lesion. Any trauma to an active lesion in the tracheobronchial tree must be religiously avoided. A biopsy should not be taken unless there is a reasonable doubt as to the tuberculous nature of the lesion. If an ulcerated tuberculous lesion is found, any nearby collection of sputum is aspirated and any sputum or exudate on the ulcerated area is gently wiped away with a cotton applicator. No effort is made to wipe off adherent exudate or membrane. The lesion is then cauterized with a freshly prepared 30 per cent solution of silver nitrate on a cotton applicator. There must be no excess drops of solution on the applicator and it must be applied accurately to the ulcerated area and nowhere else. Immediately afterwards any secretions present are aspirated and the bronchoscope is withdrawn. After the patient is returned to his room a steam inhalator is kept in operation for twelve to twenty-four hours.

This treatment is repeated at two-week intervals as long as any visible evidence of ulceration remains. We have found that intervals longer than two weeks have not been sufficient and shorter intervals have not appeared necessary. After the lesion has healed it is inspected twice at two-week intervals and then twice more at monthly intervals, since in the early period after original healing, recurrences are frequent and usually respond rather promptly if treated early. Six of our 19 patients with ulcerated lesions have had a definite recurrence after the original healing. If such a recurrence is found, the whole routine is repeated. After bronchoscopies have been discontinued any increase

in cough, recurrence of wheeze or reappearance of tubercle bacilli from a previously negative patient is taken as an indication for immediate repetition of bronchoscopy.

Dilatation of a stenotic area is never attempted until at least two months after the last evidence of an active process has disappeared, unless it should be considered absolutely essential for relief of toxaemia from retained secretions. In general, dilatation has been disappointing, both in our experience and in the experience of others (9, 11, 31, 42). We have not in any single instance been able to relieve definitely and permanently a fibrostenosis. We have not had any experience with the copper electrode of Kernan which he believes is superior to ordinary dilatation for this purpose (24).

However, periodic dilatation and aspiration of an obstructed bronchus for which no more radical therapy is feasible may be an extremely valuable procedure for some patients and may be continued indefinitely (3). We have one patient in our series, now leading a fairly active life, who before such treatment was started was a hopeless invalid and unable to walk up-stairs or more than a very short distance on the level without bringing on an exhausting fit of coughing.

RESULTS

Of the 26 patients in this series, 7 (27 per cent) are now classified as arrested (National Tuberculosis Association Classification), 16 (61 per cent) are improved, 2 (8 per cent) are unimproved or worse and one (4 per cent) is dead. Eight patients are discharged and 6 are able to work. In 5 of the 7 arrested cases sputum is negative on culture or guinea pig inoculation. The remaining 2 cases have had numerous negative microscopical examinations of concentrated twenty-four-hour sputum specimens but have not had culture or guinea pig inoculation done.

In 23 of the 26 patients the local lesion visible through the bronchoscope healed entirely. Six have the lumen of a main bronchus reduced to 3 mm. or less and 7 have the lumen of a lobar bronchus reduced to 2 mm. or less. Seven of the 25 living patients have bronchiectasis and 5 of these patients with bronchiectasis are among the 13 patients with stenosis of a main or lobar bronchus.

Eight of the 25 living patients have been under observation for six months or less since the bronchoscopic diagnosis was made. Three have been observed from seven months to one year, 4 between one and two years, 5 between two and three years and 6 between three and five years. All of the patients now classified as arrested have been observed at least two years since the bronchoscopic diagnosis was made. Seven of the 16 patients classified as improved have been observed six months or less since the bronchoscopic diagnosis was made. In 22 of the 25 living patients there is no longer any bronchoscopic evidence of active disease. In 7 of the 16 patients classified as improved the chance of the patient's disease becoming arrested appears excellent. In none of the remaining 9 and in neither of the 2 classified as unimproved or worse is the present outlook hopeless. With one exception, all of the living patients

who are not classified as arrested are still under treatment at the Sanatorium. In 4 of the 11 patients now classified as worse, unimproved or improved with a rather poor prognosis, their present unsatisfactory condition is apparently due entirely to the parenchymal disease present and not to the results of the healed bronchial lesion.

The single death in this series deserves further mention. This patient was very ill when the diagnosis of tracheobronchial disease was first made. Bronchoscopy revealed an extensive and acute lesion with ulceration on the posterior wall of the trachea from the larynx to the carina and with involvement of the left main bronchus as far as it could be inspected. Under treatment the whole lesion healed, with severe stenosis of the left upper lobe bronchus but only moderate diminution in the lumen of the main bronchus. The patient's general condition improved remarkably and she seemed to be on the road to recovery when she died suddenly from an exsanguinating pulmonary haemorrhage. Autopsy confirmed the bronchoscopic impression of complete healing of the extensive lesion of the trachea and left main bronchus. Microscopical sections through these regions showed a single submucosal, epithelioid tubercle and fibrosis and lymphatic infiltration but no caseation. Death was due to rupture of an aneurysm of the right bronchial artery produced by tuberculous necrosis of the arterial wall. Sections of the bronchus through the point of rupture showed no evidence of tuberculosis in the mucosa or submucosa.

Although it is evident that in such a small series of cases it is difficult, if not impossible, to evaluate the effect of the various factors that may influence mortality, it is of some interest to note that there is no evidence of any correlation between recovery and age of patients, the time interval between the probable onset of the bronchial lesion and the onset of treatment, or the extent of pulmonary involvement. Although in general the patients with mild bronchial lesions have done better than those with severe lesions, even in this respect the correlation is not close.

The relative value of the various methods of treatment used in this series is of interest but very little evidence is available for such evaluation. From what is generally known of the response of tuberculosis to treatment it seems almost self-evident that complete bed-rest, general sanatorium care and the judicious and skilful use of collapse therapy must be extremely important. Symptomatic treatment seems to us to have considerably more value than is suggested in the literature and to be of great importance for the patient's comfort.

We feel that in many cases local cautery of the bronchial lesions is of great importance but it is evident that its importance varies in individual cases. In those instances where the bronchial lesion is primarily a fibrostenosis at the time of diagnosis, while local treatment may increase comfort and very likely prolong life, it can certainly not be considered curative in any sense. It is also evident from the experience of others that even extensive ulcerative lesions may sometimes heal without any local therapy (2, 15, 16, 31, 33, 41). However, it is our opinion that in 19 of our 26 cases local therapy was of very considerable importance. In 2 of the remaining 7 cases which were seen early in

the course of this study, the local therapy employed was grossly insufficient by our present standards, only 10 per cent silver nitrate having been used and that at intervals of a month or six weeks. Our reasons for believing that local therapy is important are, first, that prompt improvement in the appearance of the lesion is the rule after treatment has been started; second, that in many patients whose symptoms, bacillary content of sputum and general condition had been either stationary or becoming progressively worse for many months before the onset of local treatment, improvement in all these particulars began promptly after local cautery was started; and third, both in our own experience and in the reported experience of others, the percentage of arrested cases and the percentage of deaths have been considerably more favorable when such local therapy has been adequately used.

Our results of 7 (27 per cent) cases out of 26 arrested and one (4 per cent) dead may be compared with two series collected from the literature, one in which local cautery was used (11, 13, 36, 45, 48, 50) and another in which adequate local therapy was not used (2, 5, 8, 11, 12, 41). Those from the literature in which local therapy was used showed 28 (15 per cent) of 189 cases dead and 76 (40 per cent) listed as "healed, recovered or well" (not necessarily arrested according to National Tuberculosis Association standards). Those collected cases in which local therapy was not used showed 31 (26 per cent) out of 121 cases dead and 13 (11 per cent) classified as "healed, well," etc. Combining our results with those collected from the literature in which local therapy was used shows 83 (39 per cent) of 215 cases now "arrested, healed or well," and 29 (13 per cent) dead. There is less than one chance in 2,000, considering arrested cases, and less than one chance in 200 considering deaths, that such a difference in results could be due to chance alone (Chi square method).

COMMENT

As experience with tuberculosis of the tracheobronchial tree has accumulated it has become evident, as might have been expected, that there are many analogies between tuberculosis in this location and the same disease elsewhere in the body, especially in the lung parenchyma. Thus both with pulmonary disease and with bronchial tuberculosis, symptoms are extremely variable and may be very severe or entirely absent. Physical and roentgenological findings may be very evident or entirely lacking. In both the pulmonary parenchyma and in the bronchial tree some lesions are benign and will heal without any treatment. Although we have not seen any such, there are presumably some bronchial lesions which, like some pulmonary lesions, will not heal regardless of what treatment is employed. In both conditions, it is impossible to foretell the outcome at the time of diagnosis and in both conditions recurrences after apparent healing are common.

In both bronchial and pulmonary tuberculosis, treatment used for its local effect on the lesion must be regular and persistent if it is to be successful. One may recall that, in the early days of pneumothorax therapy, some practitioners gave very large initial injections of air and gave refills only when symptoms

and physical signs recurred. Such treatment was not often successful and cautery of tuberculous bronchial lesions is likely to be unsuccessful unless it is applied regularly, at short intervals, and persistently until the lesion has healed.

SUMMARY AND CONCLUSIONS

1. The following program is suggested as a tentative but reasonable basis for the treatment of patients with tuberculous tracheobronchitis. It is offered with no sense of finality but only as an approximation to be followed until further experience suggests improvements.

(*a*) Complete bed-rest in a sanatorium and a diet adequate in all essentials.
(*b*) Relief of symptoms by a warm, humid atmosphere, adequate fluid intake, postural drainage and the use of expectorants, antispasmodics and barbiturates as necessary.
(*c*) Persistent bronchoscopic cauterization of all accessible ulcerated lesions at biweekly intervals, preferably with a 30 per cent solution of silver nitrate.
(*d*) Use of collapse therapy if the parenchymal lesion by itself demands collapse or if a main or lobar bronchus is seriously obstructed and if the obstruction cannot be promptly relieved.
(*e*) If collapse is indicated, complete thoracoplasty is usually the procedure of choice.
(*f*) In a few exceptional cases lobectomy, pneumonectomy or open cavity drainage may be used when more conservative measures have failed.

2. Twenty-six cases of pulmonary tuberculosis complicated by tuberculous bronchitis have been treated, with one death and with 7 patients now classified as arrested. A longer period of observation will probably increase both the number of deaths and the number of arrested cases.

3. In 23 of the 26 patients, the visible bronchial lesion healed under treatment.

SUMARIO Y CONCLUSIONES

1. Propónese el siguiente plan, como base tentativa, pero lógica, para el tratamiento de los casos de tráqueobronquitis tuberculosa, sin presentarlo como definitivo, sino como aproximación de lo que debe hacerse hasta que experiencias ulteriores señalen mejoras convenientes:

(*a*) Reposo absoluto en cama en un sanatorio y alimentación adecuada en todo lo esencial.
(*b*) Alivio de los síntomas mediante el establecimiento de una atmósfera húmeda y caliente, ingestión adecuada de líquido, canalización postural y empleo, según sea necesario, de expectorantes, antiespasmódicos y barbituratos.
(*c*) Persistente cauterización broncoscópica de todas las lesiones ulceradas accesibles, a plazos bisemanales, de preferencia con una solución al 30 por ciento de nitrato de plata.
(*d*) Empleo de la colapsoterapia si la lesión parenquimatosa misma la exige, o si hay una obstrucción grave de un bronquio principal o lobular, y no puede aliviarse ésta rápidamente.
(*e*) Si el colapso está indicado, la toracoplastia completa suele ser el procedimiento de elección.
(*f*) En algunos casos excepcionales, pueden emplearse la lobectomía, la neumonectomía o el drenaje de las cavernas abiertas, si han fracasado los procedimientos más conservadores.

2. En la forma descrita se han tratado veintiséis casos de tuberculosis pulmonar, observándose una muerte y estando ya siete enfermos clasificados como estacionados. Un período más prolongado de observación aumentará probablemente tanto el número de muertes como el de casos estacionados.

3. En veintitrés de los veintiséis enfermos, la lesión bronquial visible cicatrizó con el tratamiento.

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HAEMORRHAGE IN PULMONARY TUBERCULOSIS[1]

GEORGE R. MINOR[2]

Few medical phenomena are so distressing as pulmonary haemorrhage. Nothing so alarms the patient as loss of blood from some occult interior source; nothing so dismays the physician as to know that the bleeding point lies deep within a delicate organ enclosed in a rigid framework, where only slowly or doubtfully effective physiological mechanisms can be available to combat it. Whatever may be the effect on the mind, it is a matter of record that haemoptysis is usually a symptom of serious pulmonary disease. The old precept that unexplained blood-spitting is due to tuberculosis until proved otherwise is well-founded. But nontuberculous diseases such as bronchiectasis, bronchogenic carcinoma, lung abscess, rheumatic heart disease and a variety of minor nose and throat ailments are occasional causes. Particularly baffling is the patient who, apparently in good health, presents himself with the complaint of having coughed up blood recently, but shows no abnormal physical signs and few or equivocal roentgen findings. Needless to say, it is of great importance to uncover the cause of obscure haemoptysis by the use of every means at our disposal.

Despite the fairly frequent occurrence and the many observations made on pulmonary bleeding, the pathogenesis is still not entirely understood. Some ascribe it to deficiency in one of the factors concerned in coagulation of the blood (8); others to toxic (7), nervous or endocrine factors. Whether or not simple erosion of the tuberculous process through intact vessels occurs, producing haemorrhage, is still questionable. Even in the presence of a definite tuberculous cavity, the conditions which produce bleeding are not well known, though Rasmussen, as early as 1868, reported fatal haemoptyses due to rupture of a small aneurysm of a branch of the pulmonary artery within a cavity. This has since been amply confirmed; but whether all patients who haemorrhage and do not come to autopsy bleed from such aneurysms is highly questionable. Most serious haemorrhages occur in fibroulcerative tuberculosis of long standing, where much of the vascular bed of the lungs may be obliterated by endarteritis, fibrosis, cavitation and compensatory emphysema. However, many small or moderate haemorrhages occur, not in chronic lesions, but in early disease, sometimes as the initial symptom. In addition, many appear to be correlated with softening of a lesion or progression of an established process.

The data for this study were collected from the charts of 1,000 Sanatorium patients discharged in consecutive order. Only those were considered who gave a clear-cut history of expectoration of one dram or more of blood, or who haemorrhaged during their period of sanatorium treatment. "Streaking," "streaked sputum" or indefinite history of haemorrhage were not included. Of a total of 905 cases of tuberculosis, 424 were males, 481 females; among these patients there were 220 who had haemoptyses during the active phase of the disease, an incidence of 24.3 per cent, exclusive of length of observation.

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It may be noted in passing that some of the very largest haemorrhages encountered in this study were those occurring in bronchiectasis and rheumatic heart disease. Bogen (1) found more than half of his haemoptyses to be less than two ounces. These figures, however, included streaks and clots. In the series here presented there were 106 haemorrhages of stated amount, ranging from one dram to sixty-four ounces; the average size was five ounces. This did not include repeated haemorrhages from the same patient on the same or sub-

TABLE 1

Classification of cases with haemorrhage

Nontuberculous		
	Bronchiectasis	4
	Fungus disease	3
	Rheumatic heart disease	2
	Carcinoma	1
	Lung abscess	1
	Unspecified	1
Suspicious (questionable tuberculosis)		11
Pulmonary tuberculosis	Minimal	24
	Moderate	95
	Far advanced	90
Total		232

TABLE 2

The effect of haemorrhage on patients with undiagnosed tuberculosis

Local physician, diagnosis, referred to Sanatorium	103
Clinic or hospital, diagnosis, referred to Sanatorium	12
Local physician, mistaken diagnosis	9
1 called bronchitis	
1 said to have varicosities of the throat; haemorrhage and diagnosis both repeated	
3 reassured	
1 examined but not diagnosed	
1 X-rayed; not diagnosed	
1 had 5 X-ray films taken before diagnosis was made	
Patient did nothing	23
Diagnosis made only following another haemorrhage or other symptoms	
Total	147

sequent days, since these were not felt to be distinct episodes, but more or less a continuation of the first. In about 40 per cent of the cases the episode of haemoptysis was repeated at least once.

In 60 cases haemorrhage was the presenting symptom, in many instances the very first evidence of disease. The expectoration of a single mouthful of blood is much more ominous than any of the other symptoms of tuberculosis, and nothing will force the patient to seek medical advice quicker.

Seventy per cent were properly diagnosed through the activities of the local

physician when he was consulted. Eighty-four per cent probably should have been diagnosed on further observation.

As has been pointed out, the relation of the incidence of haemorrhage to the presence of cavity is direct and proportional. In this series 49 of 230 haemorrhage cases showed no cavity on X-ray examination. Conversely, of the haemorrhage patients with definite tuberculosis, 83.4 per cent had acid-fast organisms in the sputum. Of the 170 sputum-positive patients, 159 on X-ray films showed the presence of consolidation, honey-combing, punching out or frank cavitation.

TABLE 3

Haemorrhage in the absence of cavity

	NEGATIVE SPUTUM	POSITIVE SPUTUM
Nontuberculous	11	
Suspicious	8	
Minimal	7	7
Moderate	4	11
Far advanced	1	

TABLE 4

Precipitating factors

Quiet		*Exercise*	
Standing, talking	1	"Sat down"	1
Sitting in chair	1	Working	2
Awakened	3	Getting out of car	1
Trauma		Physical examination	1
Automobile accident with chest injury	4	Severe cough	2
Blow on the chest (monkey wrench)	1	Cough to dislodge peanut	2
Pneumothorax induction	1	Heavy lifting	3
Phrenic (permanent)	1	Deep breath for X-ray film	1
Phrenic (temporary)*	1		

* A medical student was discovered to have active tuberculosis with a basal cavity and a positive sputum. Right temporary phrenic nerve interruption was done, and within twenty-four hours he haemorrhaged profusely and repeatedly until pneumothorax was induced.

Whatever the exact pathogenesis of haemoptysis may be, it is a fairly common observation that it is often precipitated by physical effort or mechanical disturbance of the lungs. Many patients, of course, are awakened out of a sound sleep by haemorrhage, so we know that exertion is not a prerequisite. In fact, McCarthy (6) found that most of the haemorrhages in his series occurred in bed patients, and usually at night (1). Still, it is interesting to note the occurrence of precipitating factors and to speculate on intrapulmonary stress and strain and their effect on arterial pressure in the pulmonary circuit. In only 28 patients of this group was there any specific statement of precipitating factor or its absence.

While we cannot know the number of those with active pulmonary tubercu-

losis who have chest trauma and do not haemorrhage, the fact that haemorrhage often occurs immediately following such injury, as under "automobile accidents," "heavy lifting," "working" above, constitutes evidence that these may be precipitating agents when a cavity or caseous area of disease is present.

In 10 instances haemorrhage was related to the menstrual period. One woman had streaking and haemoptysis repeatedly during and two to three days previous to the period. Another haemorrhaged during two consecutive periods; while in a third, repeated streaking and haemorrhage occurred, most marked three or four days before the onset of the period. It did not recur when she received sodium nitrite during four days before each period began.

While haemorrhage may be innocuous, it is more often an unfortunate episode which precipitates a chain of events that may end fatally. If the haemoptysis is large and sudden, the victim may be strangled and death result from asphyxia rather than from exsanguination, although in cachectic patients blood loss may be fatal. However, the everpresent danger is that blood from a cavity which is generating a positive sputum is always apt to spread the infection to other parts of the lungs, either in the form of an acute tuberculous bronchopneumonia or as a massive caseous pneumonia. Even if a single episode of blood-spitting causes no such damage, there is the probability of repetition, which may go on *ad infinitum*.

Patients having a history of haemorrhage with early disease rarely showed any serious effect of the haemorrhage on admission to the Sanatorium. The period of softening of a caseous focus, which is marked by toxic and catarrhal symptoms, is often also marked by streaking or a small haemoptysis. Perhaps in many instances this is the best thing that could happen to the patient. With the exception of one minimal case, all those who haemorrhaged in the Sanatorium had moderate or far advanced fibroulcerative tuberculosis. Of these 36, 6 left as open cases, 2 being already moribund; 8 showed a definite spread of disease; and 12 died in the institution. Of these 12, 3 died as an immediate direct result of the haemorrhage; one instantaneously from drowning; one, three days later with interstitial emphysema of the face and neck; and one, seven days later with tuberculous pneumonia. Another died immediately following a twelve-ounce haemorrhage which was the last of a long series covering eighty-three days. A fifth died two weeks after thoracoplasty, his death apparently having no relation to the preoperative haemorrhage.

One patient haemorrhaged three, and later nine ounces from a small cavity in the right lung, and his pneumothorax refills were increased in size and frequency in an effort to control the bleeding. The increased collapse was associated with the formation of a toxic effusion which became rapidly purulent. Despite repeated tappings and irrigations, the fluid drained itself externally and became a mixed empyema. In addition to contralateral spread of disease from the original haemorrhage, a tuberculous otitis media developed, which, because of facial paralysis, required a radical mastoidectomy. Alarming postoperative haemorrhage (500 cc.) occurred several times from the wound. A few weeks later this unfortunate boy was exsanguinated by a large haemorrhage into the empyema cavity.

The remaining 6 patients died of inexorably progressive disease of which haemorrhage was only a symptom and produced no appreciable untoward effect.

SUMMARY AND CONCLUSIONS

1. In a study of 1,000 sanatorium tuberculosis patients it was found that haemorrhages occurred in 24.3 per cent of them.

2. The average size of haemorrhage was five ounces. Forty per cent of haemorrhages were eventually repeated.

3. In 60 patients, the first remarkable symptom was haemoptysis.

4. Seventy per cent of cases with a history of haemorrhage before diagnosis were properly diagnosed by the local physician, when he was consulted. However, 13 per cent were misdiagnosed.

5. Most tuberculous patients who haemorrhage have cavitation visible on X-ray examination; 83.4 per cent of this series had a positive sputum.

6. Trauma to the chest, strenuous exercise, mechanical disturbance of the lungs and, in females, the menstrual period are definite precipitating factors.

7. Small haemorrhages often occur from early lesions at the height of the catarrhal and toxaemic symptoms which probably signify softening. These are not usually serious and may, in the long run, be beneficial if they call attention to an undiagnosed tuberculosis. However, larger haemorrhages which occur in chronic ulcerative tuberculosis, while rarely immediately fatal, are accompanied by many unpleasant and dangerous possibilities. Of the 12 deaths which occurred in the Sanatorium after haemoptysis, it is felt that 5 were directly or indirectly the result of the haemorrhage.

SUMARIO Y CONCLUSIONES

1. En un estudio de 1,000 tuberculosos atendidos en el sanatorio, se descubrió que había hemorragias en 24.3 por ciento.

2. La cantidad media de hemorragias representó 150 gramos. Un cuarenta por ciento de las hemorragias se repitieron tarde o temprano después.

3. En 60 enfermos el primer síntoma notable consistió en hemoptisis.

4. Un setenta por ciento de los casos con historia de hemorragias antes del diagnóstico fueron diagnosticados debidamente por el médico local, cuando le consultaron; pero en 14 por ciento el diagnóstico fué erróneo.

5. La mayoría de los tuberculosos que tienen hemorragia muestran cavernas en el examen roentgenológico; un 83.4 por ciento de la serie tenía esputo positivo.

6. Entre los factores precipitantes bien definidos, figuran el traumatismo torácico, el ejercicio fuerte, los trastornos mecánicos de los pulmones, y en las mujeres, las reglas.

7. A menudo sobrevienen pequeñas hemorragias debidas a lesiones tempranas en el acmé de los síntomas catarrales y toxémicos, que probablemente denotan reblandecimiento. Esas hemorragias no suelen ser graves, y pueden a la larga resultar beneficiosas si hacen fijar la atención en una tuberculosis sin diagnosticar. Sin embargo, las grandes hemorragias que tienen lugar en la tuberculosis ulcerada

clínica, aunque rara vez inmediatamente mortales, van acompañadas de muchas posibilidades desagradables y peligrosas. De las 12 muertes observadas en el sanatorio después de la hemoptisis, 5 parecieron ser directa o indirectamente efecto de la hemorragia.

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SOME ASPECTS OF TUBERCULOSIS IN SWITZERLAND AT THE PRESENT TIME

F. HOMBURGER[1]

Because it may be of interest to the American physician to learn about some aspects of tuberculosis, as they are discussed in Switzerland at the present time, this compilation has been prepared from articles recently published in the *Schweizerische medizinische Wochenschrift.* It deals with the following questions.

The mingling of the rural with the urban population which occurred as a consequence of the mobilization of the Swiss Army, may be paralleled in the United States by the migration of rural workers to the industrial centres. Such intermingling of populations raises the question of primary tuberculous infection in the adult.

In the countries adjoining Switzerland the lack of adequate food has developed the problem of malnutrition in its relation to tuberculosis. Investigations of this question have led to important conclusions for the treatment of children suffering from malnutrition.

Finally, some surgical considerations with particular reference to the Monaldi treatment are presented.

THE PROBLEM OF PRIMARY TUBERCULOUS INFECTION IN THE ADULT

This is discussed by W. Loeffler (Die tuberkulöse Spät-Erstinfektion und ihre Entwicklungstendenz, Schweiz. med. Wchnschr., 1942, *72*, 686–701).

The frequency of tuberculous infection in the population has changed since Naegeli in 1896–1899 noted that among 500 routine autopsies he could prove previous tuberculous infection in 97 to 98 per cent of the cases of adults (over eighteen years of age). Clinically these findings had been confirmed by Hamburger and Monti of Vienna in large series of tuberculin tests.

During the last war, Roessle demonstrated at autopsy that only 33 per cent of German soldiers showed signs of old tuberculous infection.

Uehliger repeated Naegeli's investigations in 1933–1934, that is, one generation later. He found in 1,011 autopsies that only 80.2 per cent of the adults (65 per cent between twenty and twenty-five years of age) and 22 per cent of the adolescents (under eighteen years) yielded anatomical evidence of previous tuberculous infection. In adults from fifty-eight to sixty, there was old tuberculosis in 81 per cent of the cases.

In Zurich, Switzerland, only 50 per cent of the school children of fifteen years of age had positive tuberculin tests.

The rate of infection had been diminished, the morbidity was still high, hence the incidence of primary infection in adults had been increased.

Clinically, 5 different types of primary infection can be distinguished in the adult:

1. *The primary period of the disease in adults in general:* It can generally be

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stated that the primary tuberculous infection in the adult is either severe or mild. It often passes unrecognized.

For years, attempts have been made to find the primary focus clinically. It is certainly possible to demonstrate the complex by X-ray. With present knowledge the clinician can detect it or at least postulate its presence in numerous cases on the following basis:

The primary lymph node involvement in the child does not occur in the adult, in whom two criteria indicate the presence of a primary focus.

(*a*) A change from negative to positive tuberculin reaction.
(*b*) The transformation of a bipolar[2] soft shadow into a bipolar calcified opacity.

It is important to know the locations of the old calcified foci of the primary complex, as new softer shadows of a fresh primary infection should be sought in the same place. This localization as seen in two series of routine examinations of students of the University of Zurich is given in figure 1.

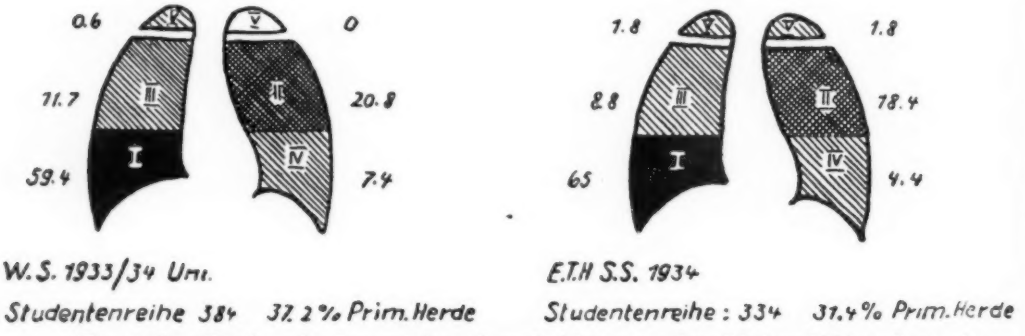

FIG. 1. Localization of primary tuberculous foci in two series of 384 and 334 students of the University of Zurich (winter 1933–1934) and the Polytechnicum (summer 1934). (Reproduced from Schweizerische medizinische Wochenschrift.)

Loeffler emphasizes that he looked for primary foci but rarely found them until he knew their probable location; then they were frequently seen.

2. *Primary infection with malignant dissemination:* Miliary tuberculosis and tuberculous meningitis in adults are secondary to a complex of primary infection. This primary focus may not be recognizable in X-ray films. Tomographic films may help in detecting them. The perifocal changes are often insignificant in adults and there may be no atelectasis surrounding the lesion.

The sizes of primary foci as described by Ghon are: 12 per cent miliary, 56 per cent the size of a pea, 23 per cent the size of a cherry and 9 per cent the size of a walnut.

Miliary tuberculosis or tuberculous meningitis in other cases of Loeffler's series was not the immediate consequence of a primary infection but the result of late dissemination.

[2] "Bipolar shadow" indicates a lesion in the lung parenchyma and one in the hilar lymph nodes.

3. *Primary infection with benign dissemination:* Erythema nodosum is considered to be a manifestation of benign tuberculous dissemination as long as no other aetiology can be demonstrated in the particular case.

Loeffler's cases include 44 of erythema nodosum. In 52 per cent of these a recent infiltrative pulmonic process could be demonstrated. In 23 per cent there were hilar changes consistent with tuberculosis. The remaining cases did not show recent tuberculous changes. However, there were sequelae of previous tuberculosis, such as pleural adhesions and old foci of dissemination.

In 10 cases, rheumatism of Poncet was found. Eight cases had exudative pleurisy before, during or after the erythema. In 3 there were phlyctenulae or episcleritis.

Reference is made in this connection to a case of simultaneous disease of 9 persons in one family from one source of infection. Six had erythema nodosum; 8 were primary infections.

It is stated that erythema nodosum must be considered as a manifestation of primary tuberculosis and must be adequately treated. Such patients should be deferred for from three to five years from military service.

However, like the other forms of generalization, erythema nodosum is not exclusively limited to the stage of primary infection. It may occur at any stage of the disease. Special conditions have to be fulfilled for its occurrence as appears from its predilection for the female sex, its frequency in northern countries and other facts.

In most of the cases a previously negative tuberculin reaction becomes positive during the erythema. When it remains negative the disease seems to belong to the group of Besnier-Boeck-Schaumann's disease.

4. *Primary infection with exudative pleurisy:* In one fourth of 109 cases the pleurisy was classified as the primary infection. This differentiation is based on the fact that the exudative pleural reaction may represent a pleural involvement in the perifocal zone.

Frequently the interlobar pleural spaces are involved and sometimes haematogenous involvement of the pleura at points distant from the primary focus is observed.

Pleurisy with effusion may well occur in a secondary stage of tuberculosis as soon as the relations between host and agent are similar to those in the primary stages of the disease.

5. Finally, *focal phthisis* of the adult is mentioned as another primary form of the disease.

ANATOMICAL FINDINGS IN PRIMARY TUBERCULOSIS

Uehliger (Schweiz. med. Wchnschr. 1942, *72*, 701), reporting on the autopsy material of the Swiss Army, concludes that the rarity of tuberculous infection in the child, due to modern hygienic measures, is responsible for the high incidence of primary tuberculous infection in the Army during the present mobilization.

Repeating Naegeli's investigations, mentioned in the preceding abstract, Uehliger found signs of previous tuberculosis in only 23.4 per cent of 707 soldiers

who had died as a consequence of violence, acute infections or coronary occlusion and whose ages varied from twenty to fifty-five years. In 65 cases, examined with particular care, this percentage was 60.

The manifestations of primary tuberculosis in the Swiss Army were sometimes as severe as those seen in the last war among colonial troops (McPherson, Leishman, Cummins: Tuberculosis history of the great war, Med. Serv. Path., London, 1923). Eighteen cases of tuberculosis occurred in one field battery during a year and a half. In this unit 2 patients with chronic tuberculosis and positive sputum were found. Fourteen of the cases were primary infections occurring in sons of large rural families in which no evidence of tuberculosis could be gathered.

Among 72 fatal cases of tuberculosis coming to autopsy from September, 1939 to December, 1941, 60 had the characteristics of primary infections, as primary pulmonic infiltration with perivascular and peribronchial lymphadenitis and caseated hilar lymph nodes.

Among 1,750 autopsies, 4 cases of primary tuberculosis had not been diagnosed clinically, because in these cases of miliary tuberculosis repeated temperature elevations had been attributed to common colds or other trivial causes.

The most common form of fatal primary infection was tuberculous meningitis which led to death in as short a time as two months.

MALNUTRITION AND TUBERCULOSIS

F. Cevey (Schweiz. med. Wchnschr., 1942, *72*, 743–745) calls attention to the fact that in Europe at the present time malnutrition is an important factor in the increase of tuberculosis (70 per cent increase in the region of Lille, France). Not only is there higher morbidity, but the disease is also more severe with predominance of the meningitic type.

The beneficial effects of food and shelter given undernourished children in Switzerland can be increased by additional tuberculin therapy.

In order to standardize his results the author used the Pelidisi formula which was applied to Austrian children by the American Red Cross in the last war. The Pelidisi factor is $\sqrt[3]{\frac{10P}{H}}$—P being body weight in grams and H the height of the sitting child from vertex to seat in cm.

In 15,000 Austrian children fed by the American Red Cross in the last war the Pelidisi factor rose in nine months by 0.9 (from 91.6).

Cevey noted that a low Pelidisi index was seen in children with positive tuberculin reaction; he treated 50 undernourished children not only with sufficient food but in addition with tuberculin. After 3.5 months of this therapy the result was an increase of the Pelidisi factor by 3.81 which means that four times as much had been obtained in half the time as compared with results in the last war with food and no tuberculin treatment.

He suggests therefore that the Pelidisi factor be checked in all undernourished children and that not only adequate nutrition be given but also tuberculin therapy.

SURGERY IN TUBERCULOSIS

In his inaugural address as Professor of Surgery at the University of Zurich, A. Brunner (Schweiz. med. Wchnschr. 1942, *72*, 781) reviewed some of the most interesting achievements in thoracic surgery and made statements concerning the Monaldi treatment that may interest readers of this abstract.

His experience with cavity drainage is large enough to allow him to evaluate its therapeutic possibilities. There is no doubt that "relatively young cavities with elastic walls may heal (with Monaldi treatment) under favorable circumstances in one to two months. In older, firm-walled cavities the treatment must be prolonged and success is uncertain. It is, however, surprising to see how the continuous suction causes the cavities to become clean. Striking improvement in the general condition of the patient is obtained, the purulent secretions are diminished in amount and in their microbic content. If the cavities are further compressed by thoracoplasty, the operation is generally well-tolerated." Brunner regards the Monaldi treatment "as a preparatory measure for the thoracoplasty, necessary for permanent success."

BOOKS

Report of the Committee on Tuberculosis in War-Time. Medical Research Council Special Report Series No. 246. Pp. 36, London, His Majesty's Stationery Office, 1942, paper, 9d. (British Information Services, 30 Rockefeller Plaza, New York, 25 cents.)

By MAX PINNER

This is a brief report on the changes in tuberculosis mortality in England and Wales and in Scotland during the three-year period of 1939 to 1941. The outstanding facts are briefly as follows:

From 1939 to 1941 the tuberculosis death rate in England and Wales has increased by 11 per cent in males and 13 per cent in females. The steepest increase is noted in the youngest age groups: from birth to five years of age it is 46 and 51 per cent, respectively for the two sexes, and from five to ten years it is 33 and 53 per cent, respectively. It is significant that tuberculous meningitis has markedly increased. Comparing 1938–1939 with 1941, the increases for England and Wales were 34 and 38 per cent, and for Scotland 37 and 60 per cent.

The factors which may be responsible for the war-time increase in tuberculosis mortality are briefly discussed and in the final chapter recommendations are made to control the situation. Among the recommendations are mentioned: roentgenological surveys, particularly in the armed forces, in industry, in nurses and medical students and in merchant seamen; more provisions for treatment; planned industrial rehabilitation; special examinations of contacts and inmates of mental hospitals; improvement in working conditions with special reference to hours of work, transportation problems, rest periods, factory canteens and control of absenteeism; extension of pasteurization of milk, since a considerable proportion of the market milk is still known to be infected; and finally suitable education of the public and the medical profession.

HARRY A. WILMER: *The Lives and Loves of Huber the Tuber. Pp. 83, with many drawings, published by the National Tuberculosis Association, New York, 1942, cloth, $1.00.*

By DAVID G. FREIMAN

Dear Huber:

I read your autobiography with great interest during one of my more or less frequent periods of insomnia and could not resist writing you about it. To begin with, you are mistaken if you think you are the last of the educated tubercle bacilli for I too can lay claim to a modicum of culture. Not all of us, it's true, have had the good fortune to read *The Magic Mountain* (my host, in spite of all my clamor, finds it rather too large a book to aspirate comfortably), but I was once coughed up in a Latin class where I managed to pick up a few things before being picked up myself. If, then, I have chosen to write this letter in Latin

rather than in that ancient combination of Tuberculosis and Sanskrit called Tuberscrit which you apparently use so freely, you will understand that it is merely with the intention of proving my point.

From the moment you slid down Doctor Wilmer's unsuspecting airway I began to worry. It's an easy descent to the primary focus as you, I and a good many humans have reason to know (the original form, *facilis descensus foco-Ghoni*, suggests that Mike must have acquired his culture in a Virgil class —[Translator's Note]), but I can't say that I think it a particularly smart idea to expose all our manifold difficulties with the immune forces and the Monosights to a human enemy who is all too ready to use such facts against us. Frankly, your little book is entirely too clear and easy to follow for my peace of mind. It is likely to prove uncomfortably useful to such laymen who want to become familiar with our strategy in fighting the Battle of Lungland and with the ways in which we can be defeated, and it is certain to be a windfall for the new patient who will, as a result, have far fewer questions to ask of the doctor. I once spent several months in a very busy larynx and I know how many questions such a patient can ask. But while none of these things is very likely to redound to our own interest, it is the illustrations in particular which are liable to prove the biggest boomerang. Excellent as they are as art, they are even better as enemy propaganda. This is total war, and if you persist in coughing up all this inside information about conditions in Lungland we are in imminent danger of waking up some fine morning to find ourselves extinct with none to mourn us.

For this same reason I was a little relieved to note the occasional liberties you took with the pathogenesis. But while I should very much like to believe that these deviations were made to mislead the enemy and confuse the issue, it appears far more likely that in these cases strict accuracy was sacrificed for the sake of dramatic effect. Even tubers, it seems, are not above that! Your idyllic but ill-fated romance with Bovy, the beauteous bovine bacillus, is not the least of these. I'm not giving away any secrets when I tell you that a romance between a human and a bovine tubercle bacillus in the same bronchus is highly improbable, to say the least. Rather than question your veracity, however, I am willing to give you the benefit of the doubt and attribute the whole thing to a natural error caused by your having only recently been rudely awakened from a long sleep in your primary focus in Tuberville and having been unceremoniously sent packing about the bronchus as a result of the New Order, not to mention the well-known and indisputable fact that Love is notoriously blind. At any rate, whatever your reasons may have been, the whole business smacks strongly of Hollywood. Nor do I think that I shall be considered indiscreet if I remark that a lymph barrier is more likely to be guarded by armed immune forces of the Monosight Defense Army than by virulent tubers. I cannot find it in my heart to blame you much for this, however, since I am a conservative tuber like yourself and have only the most profound contempt for virulent, hot-headed tubers like Nasty von Sputum, Tokiotuber, Gobbles, Gorring and others of their ilk whose sole aim it is to kill off as many hosts as possible with no thought

for the future. Any attempt made to disparage them, whether accurate or not, can, in my opinion, be nothing but commendable.

I had better close now before my own host begins to get a little anxious about all my unusual activity and starts putting those X-rays to work again. I'll toss this note out into a nearby bronchus and trust that my host will somehow manage to have it delivered to you. I sincerely hope that you will think twice in the future before attempting to write any more such dangerous exposés. You needn't both to answer this letter either, since by the time you receive it I shall probably be fast asleep again. I hope this will find you the same.

Your much-worried cousin,
Mike O'Bacterium

Lungland, 1943

WILLIAM C. BOYD: *Fundamentals of Immunology. Pp. xiv + 446, with 45 illustrations, Interscience Publishers, Inc., 1943, New York, N. Y., cloth, $5.50.*

By ERNEST WITEBSKY

Boyd's text is divided into eleven chapters; the last one is dedicated to laboratory and clinical technique. The Schick and Dick tests for instance are enclosed in the latter chapter. The author intends to give a perfectly objective, up-to-date description of immunology. In order to avoid mentioning views that have been changed or abandoned in the meantime, the subject is not approached from a historical angle. Therefore, names such as Jenner and Pasteur are barely mentioned.

The immuno-chemical nature of antigens and antibodies as well as the complicated mechanisms of antigen-antibody reactions are described superbly. The so-called "Alternation Theory," opposed by the author, is discussed in detail. Among the best chapters are those on Anaphylaxis and Allergy and Allergy and Immunity.

It is, of course, very difficult to cover all phases of immunology equally well. The immunology of specific infectious diseases, such as typhoid and typhus fever, for instance, are only shortly referred to in spite of the fact that the book contains 446 pages. Consequently, the title of the text might better be *Chemical Fundamentals of Immunology*. It will serve as an outstanding text regarding the chemical implications of immunology. It typifies an intelligent and honest approach to the involved problems of immunology, as they present themselves to-day and will have to be thoroughly studied by those who want to acquire advanced knowledge of this field.

GULLI LINDH MULLER: *Clinical Significance of the Blood in Tuberculosis. Pp. xxiii + 516, with 52 tables and 19 charts, The Commonwealth Fund, New York, 1943, cloth, $3.50.*

By MAX PINNER

How extensive the literature has grown on the haematology of tuberculosis is shown by the bibliography in Doctor Muller's book, which, although unquestion-

ably not complete, comprises 604 references. How full of controversial and conflicting statements it is, is brought out in the text. These two facts alone might warrant the writing of a book on the subject, but Doctor Muller contributes, as the most important part of the book, her own extensive and carefully analyzed observations on 1,000 patients, made over a period of five years in the State Sanatorium at Rutland, Massachusetts.

While it is, then, quite obvious that the material for a fairly large book is available, one may question whether the academic or practical importance of this special field of endeavor is pertinent enough to warrant so ambitious an undertaking. This question is simply posed; answers from competent workers will undoubtedly vary from an enthusiastic yes to a categorical no.

The first chapter deals with the physiology of the blood forming organs. A work of quite as broad a scope as this might well have deserved a more complete foundation than is provided in this brief chapter of only nine pages.

In the second chapter bone marrow studies on 74 autopsied cases are presented, indicating that in clinical tuberculosis an increased demand exists, particularly for myelogenous cells. No *intra vitam* observations on bone marrow are mentioned.

The third chapter is a brief summary of some of the literature on the response of the tissue cells to invading tubercle bacilli; this subject appears to be treated with not more than marginal interest; possibly this impression is gained because controversial opinions, which are plentiful in this field, are hardly mentioned.

The remainder of the blook is devoted to two subjects: the behavior of the cells in the peripheral blood during tuberculosis and the sedimentation of red cells. In a number of logically separated chapters, every type of blood cell is discussed, both as regards the available literature and the author's own observations. Arneth's and Schilling's methods of counting are thoroughly presented, their usefulness and limitations are shown and, in the same methodical and thorough way, various leucocytic formulae are considered. Needless to say, lymphocytes and monocytes, separately and in their alleged relation, are scrutinized in great detail. Finally, platelets and erythrocytes are discussed and, in a separate chapter, the forms and frequencies of anaemias as they occur in pulmonary tuberculosis.

Part three of the book is devoted to the sedimentation reaction; the discussions on techniques and methods for correction are particularly valuable.

Then follow a number of chapters on clinical application of haematological findings, which contain the main purpose and justification of the enormous effort that went into the making of this book. Doctor Muller's principal thesis may be summarized in the statement that serial haematological studies are most important and reliable guides in prognostication and treatment of pulmonary tuberculosis and that the one method which is of outstanding value is the sedimentation rate. In support of this dictum, the author has assembled an impressive array of data of her own work and that of many other authors. A good many more workers than are listed in the bibliography are inclined to put a value of only secondary importance on haematological data in tuberculosis.

Almost throughout the book, the reliability of haematological data is proved

by attempting to show that they mirror correctly the clinical course and pathological processes. It comes, then, rather as a surprise that in chapter 27 the justification for haematological work in pulmonary tuberculosis is derived from the fact that haematological data are statistically not highly correlated with clinical findings. "If it could be shown that a close correlation exists between the clinical data on the one hand and the hematological data on the other, the determination of only one set would be necessary. The hematological studies in tuberculosis would then add nothing but confirmatory evidence of what had already been ascertained by clinical means." It is, then, shown "that often no correlation exists."

This leads to the fundamental problem, namely, how to analyze haematological data in clinical terms. Doctor Muller has selected a few clinical phenomena in the study of correlations: weight, temperature, pulse, amount of positive sputum and pleural complications. It should be obvious that these are not the building blocks out of which a picture fully representative of the clinical course can be constructed. Whether or not a good correlation between these clinical data of limited significance and results of blood studies exist is, therefore, hardly pertinent to the discussion. And, so, the reader may well feel that convincing evidence for the clinical value of haematological data is still lacking.

In many tables and charts in which the haematological data are analyzed and presented, "tubercle bacilli in sputum" is a frequent point of reference. Of minimal cases, 15 per cent are reported as positive, 41.4 per cent of moderately and 69.4 per cent of far advanced cases. As compared to this, increased sedimentation rate is found in 55.4, 78.4 and 93.1 per cent of cases in the respective groups (table 19). According to the reviewer's experience, the positive sputum findings reported by Doctor Muller are far too low and indicative of quantitatively or qualitatively insufficient bacteriological work. I doubt that in any group of our patients increased sedimentation rates so far exceed in frequency positive sputum findings; the reverse is more often the case.

It is interesting to note in the same table that not one abnormal haematological finding (with the exception of increased sedimentation rate), even in the group of far advanced cases, reaches 50 per cent in frequency of occurrence. This may confirm the opinion of a good many laboratory workers and clinicians that bacteriological examination of the sputum produces far more pertinent diagnostic and prognostic information than do haematological methods.

The complex and frequently conflicting data and opinions from the literature are presented in well digested form; but since they are frequently confusingly contradictory, the reader would welcome, no doubt, brief summaries for some of the chapters.

One topic that should have been discussed in full detail, and that is not mentioned at all, is the unavoidable sources of errors, and their magnitudes, in counting blood cells. In tuberculosis institutions, much more often than in general hospitals, haematological work is performed without the supervision of a trained haematologist; therefore, the methodological pitfalls of this type of work need particular emphasis in a book that will be used so much in tuberculosis work.

Although one may be at variance with Doctor Muller's opinions and conclusions and even with some of her basic methods of analysis, one will recognize that her book is the result of patient and careful labor. Many workers will be deeply grateful to her, as is this reviewer, for having compiled and critically analyzed the bulk of the literature and for having presented in such clear form the wealth of her own observations. Haematologists and other laboratory workers can ill afford not to read this book; its topic is interesting and fascinating and much of it is still *sub judice*.

J. SKLÁDAL: *The Pleuro-subpleural Zone. Pp. vii + 103, with 11 plates and 20 drawings, Cambridge: At the University Press; New York: The Macmillan Company, 1942, cloth, $2.75.*

By MAX PINNER

Doctor Skládal has observed that on sudden expiration a double expiratory sound is heard over areas of cortical consolidation. This and related phenomena he studied in minute details by oscillographic and aerotachygraphic methods. The present monograph relates the experimental and clinical findings and stresses particularly the diagnostic importance of this new physical sign. The author speaks of the "autonomy of the corticopleural syndrome," but, on the other hand, he states that this syndrome may be caused by a variety of aetiological entities, such as "subacute pulmonary processes of an inflammatory or congestive nature, syndromes of subacute congestion, Potain pleuropneumonia and Grancher splenopneumonia, and syndromes of different origin, which might occur in the course of influenza, pneumococcal infections or acute rheumatism, but principally tuberculosis." The phenomenon discussed is apparently believed to be diagnostic of the "corticopleural syndrome," the pathologico-anatomical basis of which is pleural thickening and subpleural consolidation, regardless of aetiology.

The diagnostic significance of Doctor Skládal's new sign will have to be judged by further critical observation; its occurrence under conditions which the author has strictly qualified, cannot be doubted because the acoustic phenomenon was recorded both by oscillograph and by gramophone. Whether or not it serves any useful purpose to single out the "corticopleural syndrome" as a clinical entity may well be doubted.

It is not too easy to understand this monograph completely and, therefore, its just appreciation is difficult. The main difficulty is terminological and linguistic, and often it is hard to differentiate between the two. The following terms, for example, are used without definition and apparently more or less interchangeably: corticalitis, corticopleurisy, corticopleural syndrome, corticopneumonia, pleurocortical congestion, corticoscissuritis. The reader is further confused or jarred by such expressions as oedematic, cavitous, conjunctive tissue, intrabronchitic pressure, etc. Adventitious sounds are mentioned as being of "caseous, cavernous or relatively benign character." All this is not mentioned in order to

criticize, but rather in order to indicate some of the more obvious reasons why it is so difficult to render a fair evaluation of Doctor Skládal's book.

One cannot escape the impression that Doctor Skládal has lavished arduous and exacting hours of work on his thesis and that it might be interesting and possibly important to study the diagnostic potentialities of the doubled expiratory sound.

CHEVALIER JACKSON AND CHEVALIER L. JACKSON: *Diseases and Injuries of the Larynx. Pp. xi + 633, with over 200 illustrations including 11 plates in color, New York, The Macmillan Company, 1942, cloth, $8.00.*

By ALFRED A. SCHWARTZ

The Jacksons have written an outstanding contribution to the study of laryngology. Throughout the entire work, the strong personality of the authors is felt, and their contributions to laryngology are now ably condensed in the new volume.

Of necessity, the subject matters are presented briefly. Each section is concise and the presentation is extraordinarily clear. The chapter on *War Surgery of the Larynx* is excellent. The illustrations, both colored and pen drawings, are unusual, and the publishers are to be congratulated in giving a noteworthy book its splendid form.

A large section of the book deals with the surgical problems of the laryngologist, and the operative methods used by the Jacksons and others are ably and most clearly presented. This part is essentially of value to the surgeon and is unusually well written.

The chapter on tuberculous involvement of the larynx is very brief, covering only eleven pages. The clinical picture and the pathological lesions in this disease are so varied that one must regret that it is impossible to cover the subject completely in a general volume on laryngeal diseases. There are too few references on this important subject, and the names of the most notable contributors, both foreign and American, are omitted.

It seems trite to criticize a work of this magnitude. There are a number of typographical and grammatical errors which undoubtedly will be corrected in future printings. The authors, in their earnestness, permit themselves to make such statements as "drainage of serum through the intestinal tract (by the giving of laxatives) is better than attempts of local puncture," and "laxative preoperative will prevent post-operative toxic discomfort, and in laryngectomy, mental aberration."

There are other such statements, but none of them detract in the slightest from the value of the volume to the practicing laryngologist and student. It is well worth the closest attention.

W. C. HUEPER: *Occupational Tumors and Allied Diseases. Pp. xxviii + 896, 1942, Charles C. Thomas, Springfield, Illinois, fabrikoid, $8.00.*

By B. M. FRIED

The aim of the book is "to furnish an extensive source of information concerning the various aspects and the different types of occupational neoplasms which

have come to claim an ever increasing importance as a part of the problem of cancer in general."

This ambitious treatise is divided into fourteen chapters, each dealing in a thorough manner with an organ or a system affected by an epithelial or mesenchymal tumor alleged to be of occupational origin. Of all organs, the skin, as is to be expected, occupies first place—one-third of the book (about 300 pages) is devoted to this organ. To readers of the REVIEW it may be of particular interest to learn that the respiratory organs occupy a place next to the skin. The lungs, second only to the skin, come in contact more often and more intimately with occupational agents which are liable to induce neoplastic diseases. Indeed, they represent the portal of entry as well as that of an "exit" for the harmful substances that enter the body, being, so to say, a two-way thoroughfare so far as contact is concerned.

The entire section is devoted to carcinoma of the lung (Bronchogenic Cancer). All agents or factors allegedly carcinogenic for the lung are reviewed in great detail. The possible relationship between these agents and the recent statistical increase in epithelial malignant disease of the lung is discussed. To agents incriminated long since chromates are added. European observers produced evidences that chromium and its various compounds are carcinogenic for the lungs.

Physicians will hardly agree with some of Hueper's clinical and pathological utterances. He writes:

> Symptoms of lung carcinoma depend on . . . their histological character and . . . metastatic alterations in other organs.
>
> Dyspnea is an early symptom present in 90 per cent of the cases.
>
> Cyanosis and edema of the face and neck are present in 50 per cent at an early stage.

Generally, however, the book represents an important contribution to the study of occupational malignant diseases. It is of interest not only to the specialist, but to the general practitioner as well. It is well printed and is provided with an extensive bibliography.

Books Received

EDWARD W. HAYES: Tuberculosis as It Comes and Goes. Pp. 187, with 42 illustrations, Monrovia, California, 1943, fabrikoid, $2.00.

A. IBIAPINA: Pneumotorax bilateral ambulatorio. Pp. 228, with 61 illustrations, Rio de Janeiro, Gráfica Olimpica, Miguel Couto 92, 1939, paper.

DAVID O. N. LINDBERG: A Manual of Pulmonary Tuberculosis and an Atlas of Thoracic Roentgenology. Pp. xv + 233, with 189 illustrations, 1943, Charles C. Thomas, Springfield, Illinois, cloth, $6.50.

JAMES R. LISA AND MILTON B. ROSENBLATT: Bronchiectasis. Pp. x + 190, with 40 illustrations, Oxford University Press, London, New York, Toronto, 1943, fabrikoid.

DOROTHY WHITE NICOLSON: Twenty Years of Medical Research. Pp. 97, National Tuberculosis Association, 1943, paper, twenty-five cents.

DOROTHY STOPFORD PRICE: Tuberculosis in Childhood. With a chapter on *Tuberculous Orthopaedic Lesions* and other contributions by Henry F. MacAuley. Pp. vii + 215, with 87 illustrations, Bristol: John Wright & Sons Ltd., London: Simpkin Marshall Ltd., 1942, fabrikoid.

MARIO WAISSMANN: Tuberculosis congenita. Pp. 170, with many illustrations, "El Ateneo," Buenos Aires, 1942, paper.

Manual of Industrial Hygiene and Medical Service in War Industries. Issued under the Auspices of the Committee on Industrial Medicine of the Division of Medical Sciences of the National Research Council. Prepared by the Division of Industrial Hygiene, National Institute of Health, United States Public Health Service. A composite book with 16 contributors. Edited by William M. Gafafer. Pp. xi + 508, with 20 illustrations, W. B. Saunders Company, Philadelphia and London, 1943, cloth, $3.00.

The March of Medicine. The New York Academy of Medicine Lectures to the Laity, 1942. Pp. xiv + 217, New York, Columbia University Press, 1943, cloth, $2.50.

AMERICAN TRUDEAU SOCIETY

The Council of the American Trudeau Society, the Medical Section of the National Tuberculosis Association, met in St. Louis, Missouri, May 5 and 6, 1943.

In the absence of the usual business meeting of this Society, new officers and Council members were elected by ballot submitted to the membership in advance of the meeting. Eleven hundred and seventeen ballots were received and tabulated.

Officers, Executive Committee, Council members and Advisory Board members for the year 1943–1944 are as follows:

*Dr. John B. Barnwell, Mich., President
*Dr. Julius L. Wilson, La., President-Elect
*Dr. Philip H. Pierson, Calif., Vice-President
*Dr. Hugh B. Campbell, Conn., Secretary-Treasurer
Dr. Cameron St.C. Guild, N. Y., Executive Secretary

Council

Dr. J. Burns Amberson, Jr., N. Y.
*Dr. Herbert R. Edwards, N. Y.
Dr. Byron F. Francis, Wash.
Dr. R. Simpson Gass, Tenn.
Dr. Everett K. Geer, Minn.
Dr. George D. Kettelkamp, Mo.
Dr. W. H. Morriss, Conn.
Dr. Wm. H. Oatway, Jr., Ariz.
Dr. Alton S. Pope, Mass.
Dr. H. C. Schenck, Ga.
Dr. John A. Sevier, Colo.
Dr. Sidney J. Shipman, Calif.
Dr. John H. Skavlem, Ohio
Dr. Henry C. Sweany, Ill.
*Dr. George J. Wherrett, Canada

*Executive Committee.

Advisory Board

Dr. Lester Adams, Me.
Dr. Donato G. Alarcon, Mexico
Dr. John F. Allen, Neb.
Dr. G. D. Bellinger, Ore.
Dr. Henry Boswell, Miss.
Dr. Robert O. Brown, N. M.
Dr. Charles P. Cake, D. C.
Dr. H. Frank Carman, Tex.
Dr. Juan J. Castillo Arango, Cuba
Dr. Dean B. Cole, Va.
Dr. David A. Cooper, Pa.
Dr. Victor F. Cullen, Md.
Dr. M. H. Draper, Ind.
Dr. John C. Ham, R. I.
Dr. Kellie N. Joseph, Ala.
Dr. R. H. Kanable, Wyo.
Dr. Robert B. Kerr, N. H.
Dr. C. H. Lerrigo, Kan.
Dr. Paul P. McCain, N. C.
Dr. M. K. Mihran, S. D.
Dr. Lewis J. Moorman, Okla.
Dr. Edward J. Murray, Ky.
Dr. Edward J. Nagoda, Utah
Dr. Hector Orrego Puelma, Chile
Dr. W. B. Penney, Wash.
Dr. L. D. Phillips, Del.
Dr. B. S. Pollak, N. J.
Dr. Sumner H. Remick, Mass.
Dr. Jose Rodriguez Pastor, P. R.
Dr. Edward J. Rogers, Vt.
Dr. David Salkin, W. Va.
Dr. Gumersindo Sayago, Argentina
Dr. A. C. Shipp, Ark.
Dr. Frank I. Terrill, Mont.
Dr. R. D. Thompson, Fla.
Dr. Raul F. Vaccarezza, Argentina
Dr. W. L. Walbank, N. D.

AMERICAN TRUDEAU SOCIETY

The Council announces with regret the death of the following members during the year 1942:

Dr. Hugh A. Beam
Moline, Illinois

Dr. Simon P. Bittner
Glendale, California

Dr. Warren C. Breidenbach
Dayton, Ohio

Dr. Horton R. Casparis
Nashville, Tennessee

Dr. Robert M. Deming
Glencliff, New Hampshire

Dr. W. J. Dobbie
Toronto, Canada

Dr. Charles A. Dukes
Oakland, California

Dr. Julius P. Dworetzky
Liberty, New York

Dr. Jabez H. Elliott
Toronto, Canada

Dr. Carl G. Fahndrich
Battle Creek, Michigan

Dr. Libertad R. Gaetan
Rio Piedras, Puerto Rico

Dr. Marvin F. Haygood
West Point, Georgia

Dr. John H. Peck
Oakdale, Iowa

Dr. Andrew Peters
Springfield, Massachusetts

Dr. George Francis Rozelle, Jr.
Dallas, Texas

Dr. William J. Ryan
Pomona, New York